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Physiological Zoölogy

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Physiological Zoölogy

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CHICK TISSUE CELLS AND CHLORELLA IN MIXED CULTURES

(Three plates)

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Department of Zoölogy, University of Chicago

PLANT cells live side by side with animal cells in the bodies of certain invertebrates. Many species of protozoans, sponges, coelenterates, and flatworms regularly harbor green algae (Buchner, 1930, has an exhaustive list). The first extensive experimental studies on such plant-animal relationships was that on the green flatworm, *Convoluta roscoffensis*, showing that the worm lives without feeding by utilizing the carbohydrates produced by its resident green algae (Keeble and Gamble, 1907). More recently, *Paramecium bursaria* containing *Chlorella* has been the most studied example. The algae utilize the CO_2 of the paramecia, and the paramecia are able to digest the algae when other food is scarce (Parker, 1926). The alga has been grown in pure culture and identified as *Chlorella ellipsoidea* (Loefer, 1936). For *Convoluta*, for *Paramecium*, and supposedly for others, the relationship is one of mutual benefit (symbiosis or, better, mutualism). Presumably, the animal receives an increased oxygen supply, a steady removal of CO_2 and nitrogenous wastes, and an increased or emergency food supply. In most cases it has been easier to demonstrate the benefits received by the alga: a good supply of CO_2, a protected position, and a reliable source of nitrogen.

No natural mutualism of this type is known for vertebrates, but an artificial symbiosis involving chick tissue cells and *Chlorella* has already been reported in a preliminary note (Buchsbaum, 1934).

EXPERIMENTS AND RESULTS

The unicellular green alga, *Chlorella*,[1] was selected for combination with vertebrate tissue because it occurs in invertebrate tissues, it withstands relatively high temperatures and other adverse environmental conditions, and because some species grow well in aseptic culture on inorganic-salt agar medium.

Mixed cultures of algae and amphibian (salamander heart) tissue grown in diluted chicken plasma and chick embryonic extract in the light at room temperature (23° C.)

[1] A strain of *Chlorella pyrenoidosa* was used, kindly supplied by Dr. Robert Emerson, of the California Institute of Technology. Dr. Emerson's formula and the one on which the author has kept the algae growing aseptically for several years, is: KNO_3 0.5 gm., KH_2PO_4 0.2 gm., $MgSO_4 \cdot 7\ H_2O$ 0.2 gm., $CaCl_3$ 0.1 gm., $FeSO_4$ trace, agar 20 gm., hard glass-distilled water to make 1 liter.

appeared distinctly greener to the naked eye than cultures of algae alone. The colonies of algae that grew close to the tissue fragment were large, those farther from the explant were very small and pale green in color (Fig. 1). The heart fragments continued to beat for a longer time in mixed cultures than in cultures of tissue alone. Because of the relatively slow migration and growth of amphibian tissue, more detailed studies were made with chick tissues.

Cultures of connective tissue from 7-day chick embryos were made on cover slips in chick embryonic extract and heparinized chicken plasma. After several subcultures, when the growth was fairly uniform, a culture was divided into two very nearly equal parts; to one, the control culture, was added the usual embryonic extract and plasma medium; and to the other, the experimental culture, was added the extract and plasma medium containing algae. The final concentration of algae was about 30,000 per $mm.^3$ of medium. The cultures were grown in the light supplied by two 25-watt bulbs placed 10 cm. beneath the glass shelf bearing the trays of culture slides. The incubator was kept at 38° C.

Observations on the algae.—*Chlorella* in extract and plasma alone grew slowly, producing small colonies within 48 hours. The chloroplasts gradually became a pale green in color and shrunken in appearance. If the culture was opened to the air, but not allowed to dry out, the algae again became greener in color. With tissues, the algae grew much more abundantly, producing large colonies especially about the explant and the new growth. The individual algal cells were larger, and the chloroplasts were deep green. The algae far from the explant appeared less healthy than those near to the explant.

Observations on the tissues.—In an ordinary connective-tissue culture, cells migrate from the explant into the medium, dividing frequently. In an undisturbed preparation this growth continues for about 3 days and then rapidly diminishes. The cells gradually accumulate fat droplets and granules. After 5 days live cells are rare. If the culture is washed in physiological salt (Tyrode) solution and new medium is added, growth is resumed, and some of the cells can be kept alive for several weeks. The alternative treatment is subculturing. This results in a greater amount of growth and revival of activity. With a proper technique, a strain of cells can be kept alive and growing for as long as is desired.

In mixed cultures of algal and tissue cells not only was the amount of growth increased, as will be shown quantitatively below, but the cells showed other changes indicative of greater well-being. The most notable difference between tissue cells growing alone and those with algae was the almost total lack of fat droplets in the cells of the mixed cultures (cf. Figs. 2, 4, 6, the cultures without algae, with Figs. 3, 5, 7, the cultures with algae). Cultures with algae contained live cells free from fat as long as 8 days, while the controls were dead after about 5 days. Also, the cells in mixed cultures were clear and free from granules for a longer time. Old cultures containing algae produced a larger number of live cells in subcultures than did cultures without algae. The effect on the macrophages was marked. Most of the macrophages engulfed algal cells. The macrophages with algae (Fig. 3) were motile and free from fat at the same time that macrophages in control cultures (Fig. 2) were very fatty and quiescent. The number of algae engulfed varied with size and opportunity from one to twenty or more. The macrophages, which migrated from the explant first, had the greater number of algae. Within the phagocytes the algal cells were in various stages of disintegration from being quite intact to being completely broken down. Very full macrophages were less motile than those which contained only a

TABLE 1

Relative Growth of Tissue Fragments *in the Light* With and Without Algae

No.	Area of explant grown with algae mm.^{2}	Area of growth of tissue with algae mm.^{2}	Relative Increase	Area of explant grown alone mm.^{2}	Area of growth of tissue alone mm.^{2}	Relative Increase	Difference in Relative Increase
	a	b	$\frac{b-a}{a}$	c	d	$\frac{d-c}{c}$	$\frac{b-a}{a} - \frac{d-c}{c}$
1.	0.82	13.70	15.71	0.66	7.65	10.59	5.12
2.	1.49	22.04	13.79	1.08	6.63	5.14	8.65
3.	1.27	12.81	9.09	1.05	4.38	3.17	5.92
4.	1.23	3.03	1.46	1.15	1.70	0.49	0.97
5.	0.57	6.69	10.74	0.53	1.47	1.77	8.97
6.	0.57	20.20	34.44	0.52	8.68	15.69	18.75
7.	0.53	19.32	35.45	0.43	2.85	5.63	29.82
8.	0.75	9.34	11.45	0.67	6.14	8.16	3.29
9.	0.29	9.50	31.76	0.30	2.52	7.40	24.36
10.	0.50	8.92	16.84	0.60	9.57	14.95	1.89
11.	0.43	9.40	20.86	0.47	4.89	9.40	11.46
12.	0.36	9.68	25.89	0.49	3.19	5.51	20.38
13.	0.39	8.57	20.97	0.62	5.29	7.53	13.44
14.	0.90	14.25	14.83	1.11	12.18	9.97	4.86
15.	0.17	3.98	22.41	0.29	1.79	5.17	17.24
16.	0.10	4.23	41.30	0.19	1.43	6.53	34.77
17.	0.13	7.71	58.31	0.12	1.29	9.75	48.56
18.	0.75	11.60	14.47	0.62	4.54	6.32	8.15
19.	0.67	10.60	14.82	0.92	7.93	7.62	7.20
20.	1.02	15.81	14.50	1.01	7.73	6.65	7.85
Mean	0.65±0.10	11.07±1.15	21.45±2.88	0.64±0.07	5.09±0.69	7.37±0.84	14.08± 2.67

TABLE 2

Relative Growth of Tissue Fragments *in the Dark* With and Without Algae

n = 20	Area of explant grown with algae mm.^{2}	Area of growth of tissue with algae mm.^{2}	Relative Increase	Area of explant grown alone mm.^{2}	Area of growth of tissue alone mm.^{2}	Relative Increase	Difference in Relative Increase
Mean	0.17±0.04	2.47±0.54	14.13±3.11	0.16±0.03	3.76±0.83	23.11±5.08	-8.98±1.97

TABLE 3

Relative Growth of Tissue Fragments With Algae *in the Light* and *in the Dark*

n = 15	Area of explant grown with algae *in the light* mm.^{2}	Area of growth with algae *in the light* mm.^{2}	Relative Increase	Area of explant grown with algae *in the dark* mm.^{2}	Area of growth with algae *in the dark* mm.^{2}	Relative Increase	Difference in Relative Increase
Mean	0.98±0.07	4.97±0.61	4.17±0.66	0.94±0.07	3.79±0.53	3.11±0.56	1.06±0.28

few plant cells. Most of the phagocytosis took place during the initial migration of macrophages during the first day of growth, although more algae-laden cells were seen in older cultures than in young ones. Fibroblast-like cells occasionally took up algae.

The details of the phagocytosis have been recorded in color by means of cinematography.[2] The algae are approached by apparently random movement of the phagocytes. When the undulating membrane of a macrophage comes into contact with an algal cell, the protoplasm appears to flow around and in contact with the alga, which then sinks suddenly into the cytoplasm. There is no surrounding with pseudopods, as in some types of phagocytosis. As digestion proceeds, vacuoles form about the inclosed algae (Fig. 3).

Besides the cultures made for the observations described above, three series were made for quantitative analysis of growth: chick tissue with and without algae in the light, tissue with and without algae in the dark, and tissue with algae in the light and in the dark. In these growth experiments the explants of both experimental and control cultures in each pair were derived from a single symmetrical culture. The explants were projected and their outlines drawn shortly after the cultures were made and again after 48 hours (Buchsbaum and Loosli, 1936). The areas of the outlines were determined with a planimeter and computed to square millimeters. Fifteen to twenty pairs of cultures were thus compared for each of the three series. The area of growth after 48 hours minus the area of the original explant is the area of absolute increase. The area of absolute increase divided by the original area of the explant is the relative increase. The differences between the relative increase of the experimental and the control cultures were treated statistically (by Student's method). In Tables 1, 2, and 3 the standard errors of the means are given.

In the light, the cultures of tissue with algae grew significantly better (probability = 0.0000) than the cultures of tissue without algae (Table 1). In the dark, the cultures of tissue grew significantly better (probability = 0.0007) than the cultures of tissue with algae (Table 2). The tissues with algae in the light grew significantly better (probability = 0.0020) than the cultures of tissues with algae in the dark (Table 3).

DISCUSSION

Plant cells.—When light was available, the algal cells grew better in the plasma-extract medium in the presence of tissue than in its absence. Three possible factors contributing to the beneficial effect on the algae by the tissue will be considered: (1) the tissue could supply nitrogenous substances for protein synthesis; (2) it could "condition" the medium by removing some toxic substance; or (3) it could supply carbon dioxide for photosynthesis.

Direct measurements for nitrogen have not been made; however, it is known that the tissues release nitrogenous substances into the medium. Other workers have emphasized the exchange of nitrogenous substances in symbiotic relationships. Lipman and Teakle (1925) maintained an artificial symbiosis between *Chlorella* and *Azotobacter chroococcum* and reported that the algae in the mixed cultures were distinctly greener than the algae grown alone. Here, significantly greater quantities of nitrogen were fixed by bacteria growing with the algae than by those growing alone. Hence, greater quantities of nitrogenous compounds were available to the algae in the mixed cultures. Keeble and Gam-

[2] The moving pictures were kindly made by Mr. P. S. Tice, Chicago, Illinois.

ble (1907) suggested that nitrogen supply is the basis of the widespread alga and marine invertebrate symbiosis, which is exceedingly common in tropical waters, where the nitrogen content is low, and infrequent in colder waters, where the nitrogen content is much higher. In the open ocean, CO_2 is available in large quantities and does not act as a limiting factor for photosynthesis; but in a small, sealed drop culture, rich in nitrogenous substances, the supply of CO_2 can easily become the most important limiting factor. Also, in cultures of algae with tissue in the dark, the algae are not perceptibly improved over those grown alone in the dark, so that the nitrogen supply cannot be the most important factor accounting for the improved condition of the algae.

Another possible factor is the action of the tissue in "conditioning" the medium by removing a substance toxic to the algae by some mechanism like that involved in the mass protection afforded by increased numbers of organisms in toxic solutions (Allee, 1931). However, any protection afforded by the increased number of organisms cannot be the most important factor acting, since it exerts no perceptible effects in the dark. The nitrogen and the "conditioning" factor, both of which could operate in the dark, were not enough to account for the increased growth of the algae with tissue in the light. It would appear, therefore, that the increased growth of *Chlorella* in the presence of tissue in the light is related chiefly to the carbon dioxide furnished by the tissue.

Animal cells.—When grown in the light, chick tissue cells do better in the presence of algae than in their absence (Table 1). This could be due to any or all of several factors. The algae could furnish a direct source of food, remove nitrogenous wastes or other substance harmful to the tissue, supply O_2, or remove CO_2.

The utilization of algae as a direct source of food cannot be considered to be the major factor, since tissue cells actively phagocytized algae in the dark as well as in the light. Further, the amount of food digested could not be available to the entire culture, and most of the fibroblasts did not phagocytize. The removal of nitrogenous wastes could go on to some degree in the dark, and the conditioning factor in removing toxic substances (other than CO_2) should be little affected by the lack of light. Hence, these cannot be considered to be more than contributory factors.

The increased supply of O_2 and the steady removal of CO_2 (and the consequent change in H-ion concentration) would remain the only two factors which would be completely reversed by the change from light to dark. In the dark, the algae would add CO_2 to that given off by the tissue and would compete with the tissue for O_2. This situation would be enough to mask any benefits that the tissue would derive from any of the foregoing factors or even the possibility that algae which are actively photosynthesizing would add glucose to the medium. The results (Tables 2 and 3) are in agreement with this consideration.

The preliminary report of this work (Buchsbaum, 1934) suggested to Gause (1935) that such an artificial symbiosis [mutualism] as here described might supply the needed experimental data to substantiate the theoretical curves of growth of a mixed population of two mutualistic organisms. Unfortunately, the kind of data required for such population studies is not readily obtained from algae-tissue culture experiments because of the difficulties in counting the algae and in estimating the growth of the tissue fragments. However, an artificial mutualism between *Azotobacter* and *Chlorella*, or between root tips and *Chlorella* in fluid medium should be excellent material because both species could be accurately counted or measured and precise growth curves obtained.

SUMMARY

1. Mixed cultures of embryonic chick connective-tissue cells were made with the green alga, *Chlorella pyrenoidosa*, in chick embryonic extract and chicken plasma medium.

2. In the light, the algae grew better in association with animal cells than control cultures of algae alone.

3. In the light, the tissue cells with algae grew better and remained alive and free from fat longer than tissues alone.

4. In the dark, tissues without algae grew better than those with algae.

5. The data are best explained on the basis of a photosynthetic mutualism.

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PLATE I

FIG. 1.—Amphibian (salamander heart) tissue with algae after 10 days' growth in the light. The large colonies of algae can be seen covering the explant; only very small colonies are seen away from the explant. ×100.

FIG. 2.—Macrophages in chick tissue culture grown without algae for 5 days in the light. Note the large number of fat droplets in the cells and their absence in the cells in Fig. 3. ×635.

FIG. 3.—Macrophages in chick tissue culture grown with algae for 5 days in the light. ×635.

PLATE II

FIG. 4.—Chick connective tissue culture grown without algae for 5 days in the light. The cells are degenerate and contain abundant fat droplets. ×635.

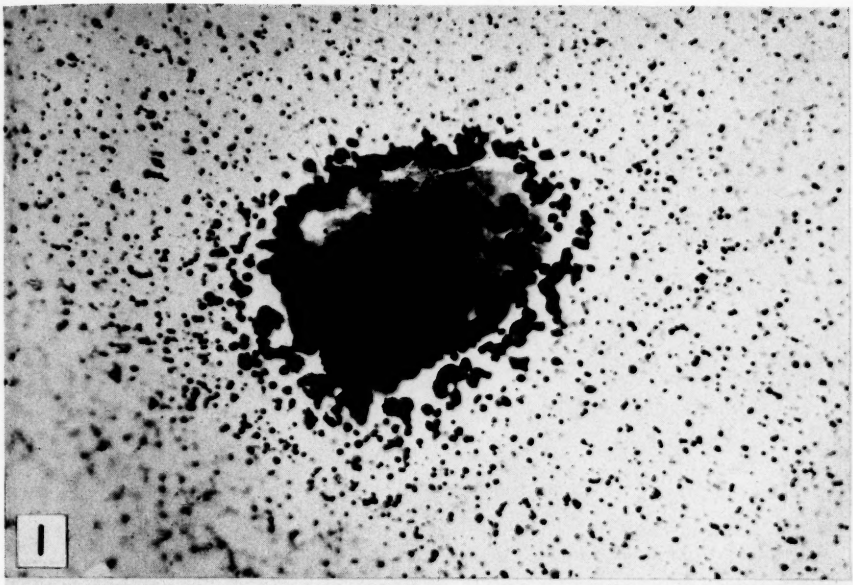
FIG. 5.—Chick connective tissue culture grown with algae for 5 days in the light. Most of the fibroblast like-cells are alive; fat droplets are rare. ×635.

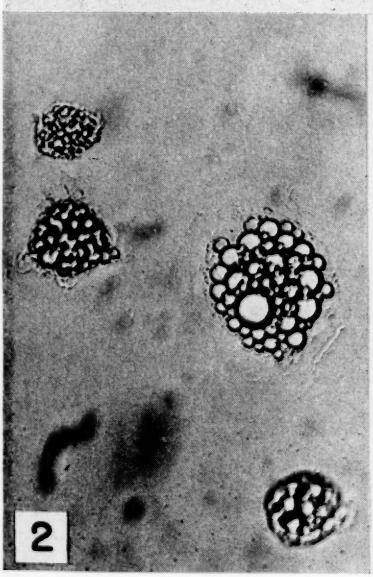
PLATE III

FIG. 6.—Chick connective-tissue culture grown without algae in the light for 8 days. Most of the cells are dead; cells are packed with fat droplets. ×635.

FIG. 7.—Chick connective-tissue culture grown with algae in the light for 8 days. Note the abundance of algae, the finely granular, rounded appearance of the cells, and the general absence of fat droplets. ×635.

PLATE I





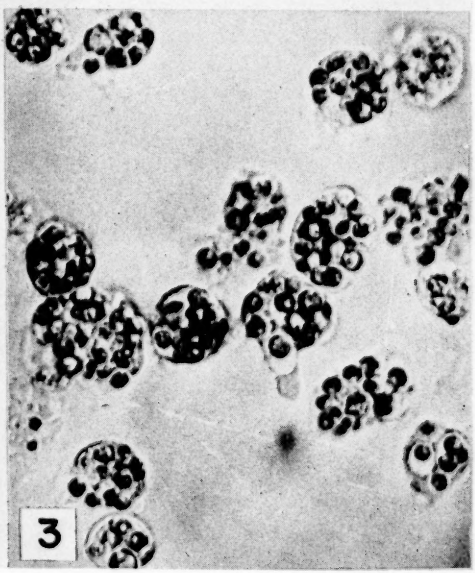


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PLATE II

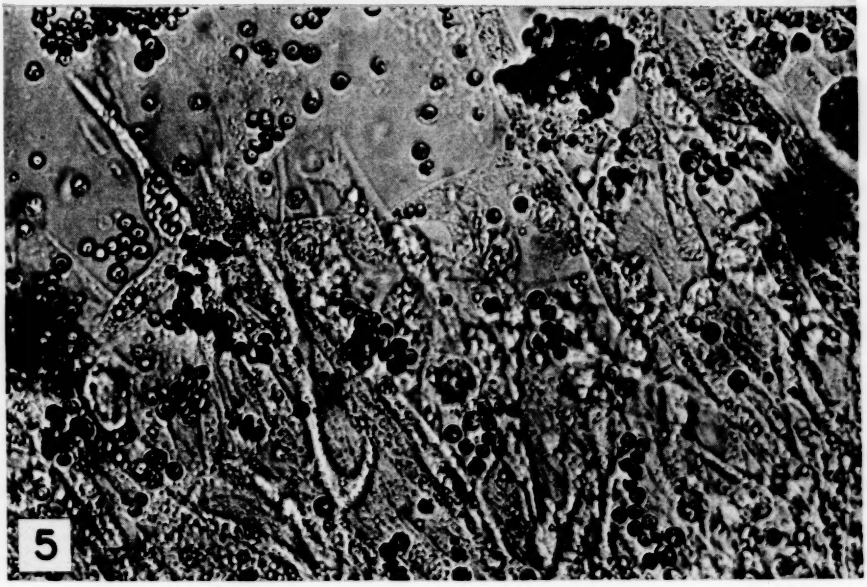

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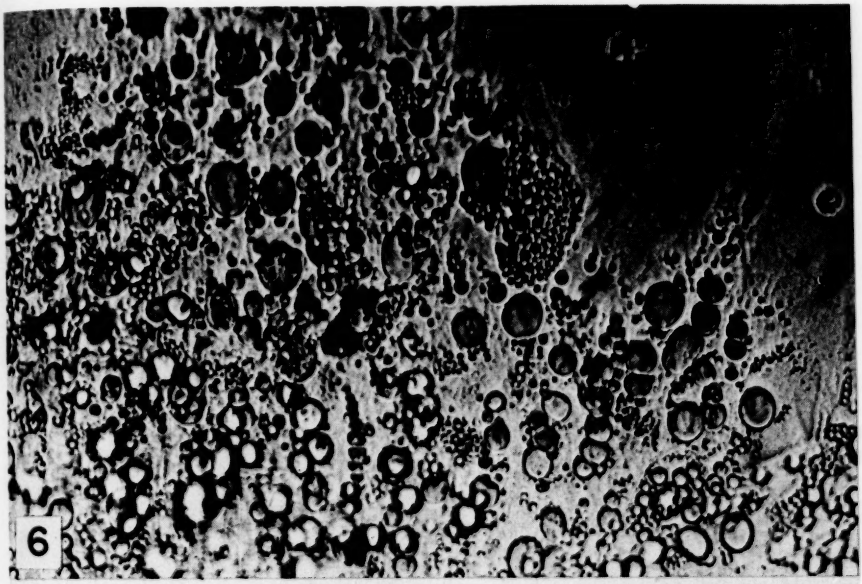
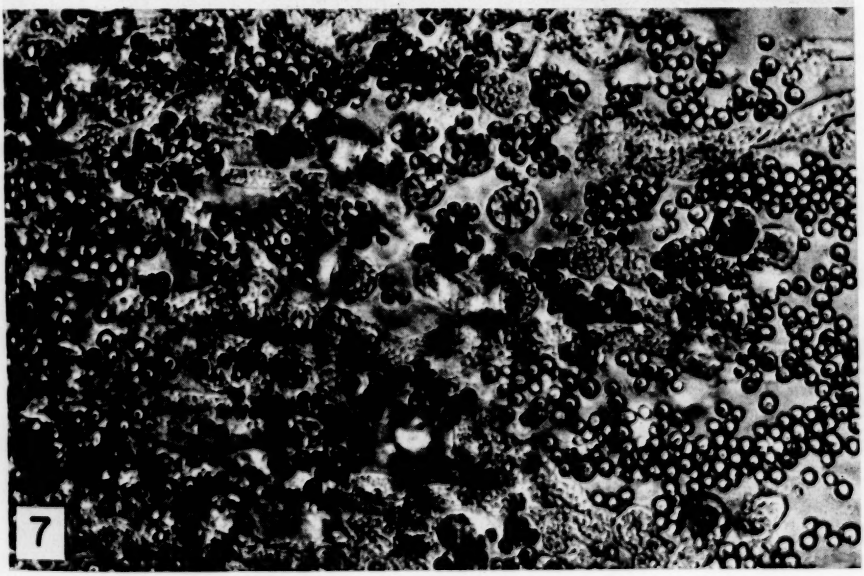
PLATE III





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DIFFERENTIATION OF THE GUT IN CHORIO-ALLANTOIC GRAFTS FROM CHICK BLASTODERMS

(Fifteen figures)

DOROTHEA RUDNICK AND MARY E. RAWLES
Department of Zoölogy, University of Rochester

THE problem of the differentiation of the embryonic gut of the chick in chorio-allantoic grafts is one that has never been adequately discussed. Interpretations and descriptions of individual cases are frequent in the literature (Hoadley, 1925, 1926; Murray, 1928; Willier and Rawles, 1931; Hunt, 1931; Rudnick, 1933; Dalton, 1935; Butler, 1935; Rawles, 1936), but no general survey has been made. During the past few years both of the authors of this paper have given considerable attention to problems concerning the early localization and developmental mechanics of the entodermal glands; in the course of these and other investigations we have examined a number of grafts of early blastoderms (primitive streak and head-process stages) and of

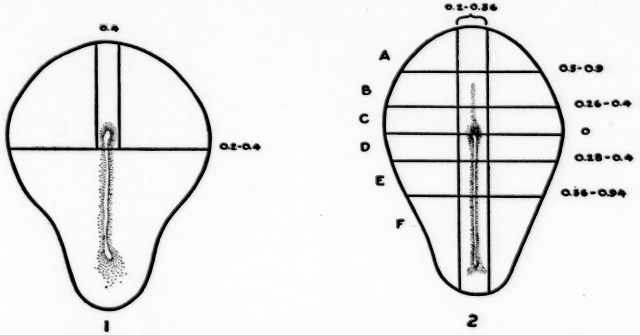

FIG. 1.—Diagram of cuts made on primitive streak blastoderm. Distances from pit given in millimeters.

FIG. 2.—Diagram of cuts made on head-process blastoderm.

measured pieces of these blastoderms, and have come to a few definite conclusions concerning the localization on the blastoderm of potencies for formation of the gut tube itself as well as concerning the differentiation capacity of potential gut material in the graft environment. It has seemed worth while to pool our information in the present report.

The experimental material on which this study is based has for the most part been described in other connections. The graft series comprise: (1) Grafts of the definitive primitive streak blastoderm (see Fig. 1), cut into four pieces (Rudnick, 1932). (2) Grafts of the entire primitive streak blastoderm from which the entodermal layer was completely or almost completely removed (Rawles, unpublished). (3) Grafts of one-eighteenth pieces of the head-process blastoderm (Rawles, 1936). For the present purposes these grafts will be described as belonging to the anterior embryonic field (level *B*), the node field (levels *C* and *D*), or the streak field (level *E* and the anterior of level *F*) (see Fig. 2). (4) A small series of grafts in which the gut of embryos from 14 to 30 somites

was divided into segments and transplanted, usually along with adherent surrounding material (Rudnick, unpublished).

The grafts were grown for 8–10 days on the host membrane and subsequently examined in serial section. Throughout, the gut tissues of normal embryos of corresponding age have been used for comparison.

We are much indebted to Miss Marion Leffler for the execution of the text illustrations.

1. DIFFERENTIATION OF GUT TUBES IN GRAFTS

If one considers the nature of the developmental process in grafts, which tends to analyze structures into deformed masses of their component tissues, it is perhaps surprising that a complex organ such as the gut should ever attain anything like its normal form under such conditions. Even in transplants of the already formed gut tube, it is not often that differentiation proceeds without gross mechanical distortion, suppression or hypertrophy of constituent parts, and especially, as will be described in a subsequent section, marked irregularities in the epithelial pattern.

However this may be, examination of head-process and primitive streak grafts shows fairly numerous cases where true gut tubes have differentiated, as well as the almost universal occurrence of their constituent tissues. The gut tubes are, of course, subject to the same variations in form exhibited by grafts of the closed gut. In the best cases they present cross-sections indistinguishable from that of the normal gut except for size (Figs. 13 and 15).

Head-process grafts.—The regions of the head-process blastoderm capable of undergoing organized differentiation can be accurately determined by means of the eighteen-piece series (Fig. 2). In respect to gut differentiation, the blastoderm at this stage divides itself into two distinct regions. The anterior embryonic and node fields, distinguished by a capacity to form axial structures both in normal development and under experimental conditions, have only very diffuse capacity to form gut. Grafts from this region contain, among many other tissues, epithelia characteristic of all levels of the gut, with or without surrounding smooth muscle, all in the most disorderly profusion. Rarely, in these levels, true gut tubes are formed; but isolated instances have been observed scattered throughout. In contrast, the principal component of grafts of the streak field is intestinal tube. These tubes are distinguished by the normal picture they present and by the fact that they may grow suspended in the interior of vesicles as if in coelomic cavities (Fig. 13). In grafts from this region the only unorganized epithelia produced are those characteristic of the intestinal level. To supply concrete figures, out of a total of 120 grafts from the anterior embryonic and node field there were 7 cases of structures approximating the form and arrangement of organized gut—about 6 per cent. In 65 grafts from the streak field, 13, or 20 per cent, contain organized gut—the best cases far exceeding anything found in the anterior part of the blastoderm as regards organization. The rise is more striking than the figures indicate, since general developmental potency is low in the posterior region of the blastoderm (Rawles, 1936).

The seven cases in which true gut tubes were formed in grafts, including parts of the anterior and node fields, are of some interest in that they show that organization factors for the gut are not entirely limited to the streak region. For this reason they will be described briefly in this place.

Case 1 (Fig. 3).—The gut in this case appears as a short segment completely imbedded in host mesenchyme. Two definite, though incomplete, muscle coats are present. The submucosa

is a definite and vascularized layer. The epithelium is of a deep, pseudostratified sort, not capable of identification as to level, and the lumen is somewhat branched. The thinness and discontinuity of the muscle coats suggest the appearance of normal esophagus; however, no certain identification of level can be made.

Case 2 (Fig. 4).—In this graft the gut tube in question is partly cut off from other structures by bilateral clefts lying on either side, parallel to it for a short way (only one illustrated). This adds to a general resemblance to incompletely differentiated proventriculus. Incomplete circular and longitudinal muscle coats are present as well as a dense vascularized submucosa. The epithelium is of a deep, pseudostratified sort, similar to that of the 7-day normal proventriculus. One or two epithelial pouches extending into the submucosa are the only indications of gland formation. This structure is certainly well-formed gut and is very probably a segment of the proventriculus.

3

4

FIG. 3.—Drawing of section through gut tube in Graft 250–3- B_1, from the median segment of level *B* (Fig. 2). See text, Case 1. ×50.

FIG. 4.—Drawing of section through gut (proventriculus?) in Graft 252–3- C_1, from a median piece of level *C*. See text, Case 2. ×50.

Case 3 (Fig. 5).—This graft contains very typical stomach, imbedded in host mesenchyme. There is a definite ventricular region, with strikingly normal epithelium, a submucosa denser and more vascular than normal, and a heavy muscle coat which does not correspond in details of arrangement to the normal ventriculus wall. At one end of this tube the epithelial sac projects from its muscular tunic, and here the epithelium takes on an irregular, though still distinctly differentiated, appearance. At the other end the muscle coat becomes thinner and the epithelium takes on an undifferentiated, thick, pseudostratified character, a few diverticula are present. It is possible that this latter area represents proventriculus. The whole structure is much more extensive than the two cases previously described; its length is 132 sections of 7μ, or almost a millimeter.

Case 4 (Fig. 7).—In this graft is found an extensive distorted gut complex imbedded in mesenchyme. In some places, as in the region illustrated, a thick circular muscle layer is present like that of the normal small intestine. The epithelium is thrown into irregular folds by masses of

erythrocytes beneath it; its cells are vacuolated but characteristic of small intestine in height and form. Elsewhere in the complex the epithelium grades to pseudostratified.

Case 5 (Fig. 8).—This graft contains a short distorted gut tube with muscles and epithelium characteristic of small intestine. The submucosal layer is either absent or infiltrated with erythrocytes.

Case 6 (Fig. 6).—In this graft a segment of colon tube shows, for part of its length, entirely characteristic structure. The epithelium is typical, as is the submucosal condensation of mesenchyme. Especially interesting are the circular muscles, which at one point are directly continuous with a large mass of heart muscle. The whole tube is some 375μ in length.

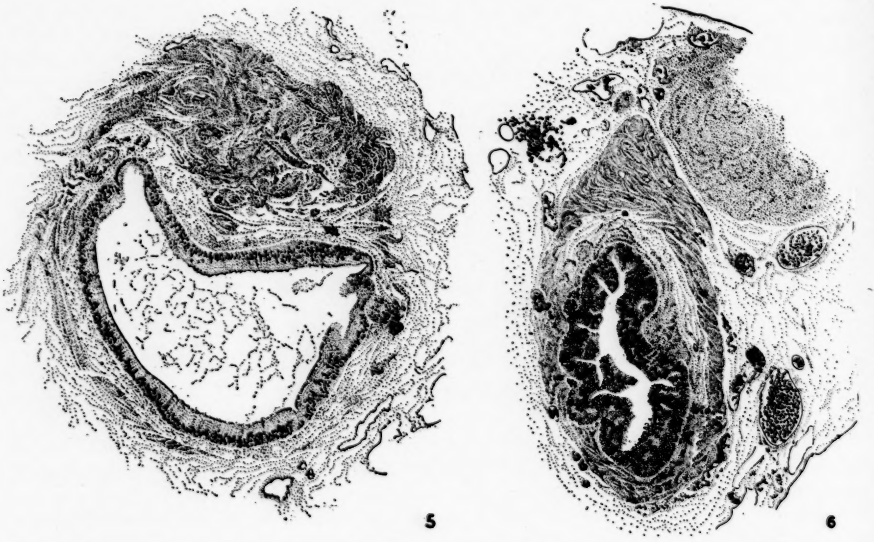

FIG. 5.—Drawing of section through ventriculus found in Graft 250–2- D_1, from a median piece of level *D*. See text, Case 3. ×50.

FIG. 6.—Drawing of section through colon formed in Graft 261–2- C_3, from a right lateral piece, level *C*. Above is a mass of heart muscle, which becomes directly continuous with the gut musculature in subsequent sections. See text, Case 6. ×50.

Case 7.—This case is also one of very typical colon about 325μ long, in part of which the lumen is occluded as in the normal rectum. The submucosa and both muscle layers are typically formed. The only abnormal feature is local amassing of erythrocytes in the submucosa.

The first case described shows that gut-forming factors are not entirely absent from the most anterior level of the axis. The rest show that it is possible for specific segments of the gut-stomach, small and large intestine, to develop in grafts of small pieces of the node level. Equally striking is the fact that such normal-appearing tubes occur very rarely, although, as has been said, their epithelial and muscular components are very frequent in grafts from this region.

Posterior to the node field is the region which, when transplanted, not only forms perfect intestinal tubes in a good percentage of cases but may form a coelomic cavity in con-

nection with these. This intestine-forming field extends at least as far back as 0.9 mm. posterior to the primitive pit, since well-formed intestine (both large and small) is found

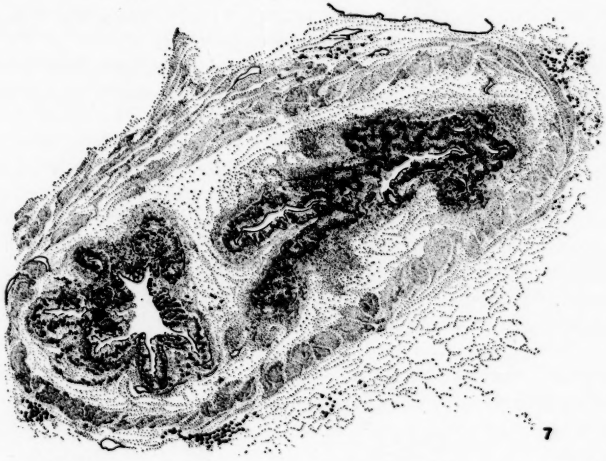

FIG. 7.—Drawing of section through small intestine complex, Graft 263–4- *C*2, from left lateral piece, *C* level. See text, Case 4. ×50.

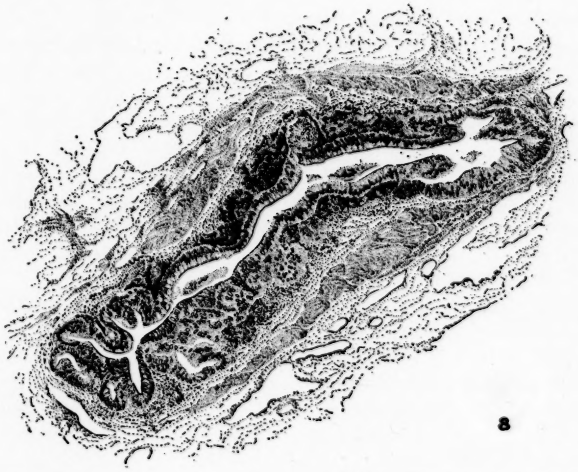

FIG. 8.—Drawing of section through small intestine complex, Graft 256–61- *D*1, from median piece, D level. See text, Case 5. ×50.

in grafts of pieces posterior to this level. In the posterior regions of this field gut is formed in median pieces only, whereas in the level from 0.3 to 0.7 mm. behind the pit

lateral pieces as well have this potency. As a matter of fact, this posterior field coincides very well with the region of prospective lateral-plate mesoderm, as projected from the scheme of Wetzel (1929), assuming that lateral plate lies peripheral to somite mesoderm.[1]

As an example of differentiation of intestine in grafts from this region, Figure 13 shows a section of an intestinal tube which is looped in a large cavity. For comparison, Figure 15 shows a section of normal 10-day small intestine. The graft tube is more deeply folded, although smaller in size. One end of the tube in the graft is equally well-differentiated large intestine.

Primitive streak grafts.—In the primitive streak stage, grafts show the same degrees of differentiation of stomach and intestinal tubes, ranging from nearly perfect to badly distorted, though still recognizable, organs. Stomach has been found in two cases; these were both grafts from anterior median pieces (consult Fig. 1). Intestine occurs rather more frequently. Out of a total of 84 grafts, organized intestine is present in 12 cases, or 14 per cent. These cases are found about evenly distributed in all four sectors of the blastoderm tested. Although this series of grafts does not give a complete localization picture, as does the more extensive head-process series, a few definite facts may be ascertained by means of the measurements obtained at the time of transplantation.

Twenty-one grafts were of anterior pieces, the posterior limits of which were less than 0.2 mm. behind the primitive pit. Of this group only one (5 per cent) contained intestine. In the remaining larger group of grafts, including posterior as well as anterior pieces, the transverse cut was made at a distance of 0.2–0.4 mm. posterior to the pit; that is to say, a portion of the material between these levels was invariably present. In this group 11 cases (17 per cent) of organized intestine occurred. In 3 of the cases the piece forming well-organized intestine was an anterior median one with its posterior limit 0.24 mm. behind the pit; the others were cut slightly farther back. These 3 cases, and the single case where an anterior left lateral piece cut only 0.12 mm. behind the pit contained organized large intestine, serve to show that at this stage the intestine-forming field has its region of high frequency at a streak level more anterior (in absolute measurement from the pit) than the corresponding region in the head-process stage; exact anterior and posterior limits cannot be ascertained from the present data. Relatively, the high-frequency region is even farther anterior than the absolute measurements indicate, since at this stage (definitive primitive streak) the streak is at its maximum length, whereas it shortens in the head-process stages. In the latter stages, as has been said, the maximum intestine-forming field is distinctly posterior to the node field; in the definitive primitive streak stage the two fields overlap.

It must be pointed out that the graft series from which these comparisons are drawn are really not exactly comparable. The main difference is one of size of piece transplanted. It will be recalled that in the head-process series 18 pieces were taken from each blastoderm; in the primitive streak series, only 4. It is our belief that the size of transplant has in itself an influence on organization in the graft; and it is possible that the larger size of the pieces in the primitive streak series may favor gut organization and so may in itself account for the seeming shift of the field. However, the percentage of posi-

[1] The recent work of Pasteels (1936), in which the localization scheme of Wetzel is criticized, would not seem to provide a basis for a lateral plate field narrowing and becoming median in its posterior portion. It is to be regretted that the very interesting results of the former author have not been reported in the same detail as those of Wetzel; nevertheless it is at least clear that the formation of the posterior regions of the embryo is much less understood than is the morphogenesis of the head and anterior body.

tive cases is alike in both groups—even a point or two lower in the primitive streak series (17 as compared with 20 per cent, to cite only the figures for the intestine-forming field in both cases), so that size differences in the transplant have certainly had no effect on frequency of intestine formation. In any event, the data for the primitive streak blastoderm do not show the correspondence between area of maximum intestine-forming potency and position of lateral-plate mesoderm (see Wetzel, 1929) that was so striking in the later head-process stage.

This completes the account of what may be thought of as true gut tubes in grafts of pieces of the young blastoderm. In addition to such recognizably normal structures, a tremendous variety of epithelia with and without characteristic surrounding mesoderm (muscle, cartilage, condensed or vascularized mesenchyme) occur throughout (see Fig. 14). These have been examined and classified; the results indicate mainly that embryonic epithelia are so plastic in behavior and structure that "differentiation" is scarcely a proper word to use in describing their patterns. Nevertheless, since cellular pattern is our only criterion of the early stages of epithelial differentiation, and has thus been widely used, it seems worth while to describe and to evaluate the forms found in grafts, in terms of the patterns found in the gut lining of the normal embryo at a corresponding age.

2. DIFFERENTIATION OF COMPONENT TISSUES IN GRAFTS

Inspection of the epithelial patterns in the 10-day embryo makes it clear that the basis of early differentiation varies from case to case. The gut lining at all levels shows a common tendency in its early history to thicken from a flat to a regular cylindrical-pseudostratified epithelium; from this point on, definite regional differences prevail. In the pharynx and esophagus the pseudostratified epithelium becomes stratified in two layers. In the main respiratory tubes it remains single-layered, but becomes very irregular, both as to height of cells and as to their continuity at the distal border. In the large intestine a somewhat similar cell arrangement ensues. Here, especially in the middle portion, clumps or blocks of the cylindrical cells remain adherent to one another while evidently pulled apart from neighboring cell groups; in addition, the posterior end of the tube shows hypertrophy of the primitive epithelium to the point of almost complete occlusion of the lumen (Fig. 10). In all these cases the differentiation depends not on individual cell function but on cell arrangement—that is to say, mechanics of a whole epithelial tube, and on mutual adhesion of the cells involved.

The stomach and small intestine linings, on the other hand, present pictures depending partly on cell arrangement and individual cell volume, but also largely on the appearance of a definite and polarized secretion pattern in each cell of the primitive cylindrical epithelium. These regions, then, possess differentiation on a cellular as well as on a tissue level.

Among the epithelia found in the early blastoderm grafts are represented all the types seen in the normal 10-day gut, together with intergradations, variations, and arrested forms, of which the cylindrical-pseudostratified stage through which all epithelia appear to pass is especially predominant. In the present section six epithelial types from the normal gut are discussed with reference to their appearance in grafts. These have been chosen largely because of their suitability for identification in grafts, and because they represent all levels of the digestive tube.

It should be stated that many of these epithelia, as judged by their relations in grafts

and also by their occurrence in grafts of blastoderms from which the entoderm was removed before transplantation, are ectodermal or even mesodermal in origin. When in a graft known to have contained entoderm a tube or vesicle of epithelium characteristic of a certain gut region is found to be surrounded by connective tissue and muscle masses, it would seem proper to interpret the structure as gut, even though the total picture is extremely distorted, and to believe the epithelial component to be derived from primitive entoderm. Nevertheless, complexes of this sort occasionally are found in grafts which contained no entoderm originally; it is evident that epithelia from other germ layers may not only take on a form attributable to gut lining—which is, after all, not a highly specific differentiation—but may be associated with connective tissue and muscle in gutlike complexes. Some of these will be described in their place in the following section.

1. *Pharyngeal epithelium.*—The epithelium of the 10-day pharynx is not distinguishable from skin of the same age, although the history of the two structures has differed considerably. The pharyngeal epithelium has been transformed to a two-layered stratified sort, with the external layer much flattened, after having passed through a moderately tall, cylindrical, pseudostratified stage. In grafts from blastoderms, tubes of this skin-pharyngeal epithelium may be surrounded by cartilage nodules. This is an arrangement characteristic rather of respiratory tubes; with which, indeed, the pharynx-type epithelium frequently is continuous. It is only when there is continuity with other gut tubes or glands that it has been possible to interpret this epithelium as pharyngeal rather than epidermal in grafts containing both rudiments.

In grafts from the formed branchial foregut (18–30 somite stages) typical pharyngeal epithelium is found to invest large mesenchyme or cartilage masses. Hence it is possible for pharyngeal entoderm to pursue its differentiation normally in grafts. It does not necessarily do so, as is proved by the occurrence in these foregut grafts of local patches of tall columnar epithelium, and even of stomach type (see below).

2. *Esophageal epithelium.*—The esophagus and crop of the 10-day embryo also possess a two-layered stratified epithelium. The distal cell layer shows no sign of flattening; the general arrangement of cells varies in regularity from region to region. There is no morphological evidence of secretory activity on the part of the cells, or of other polarization. Altogether, the epithelium appears remarkably unspecialized and, one would expect, capable of realization frequently under experimental conditions. As a matter of fact, such epithelial patterns are among the most rare and least stable ones occurring in grafts. Even in transplants of the closed-off esophageal region (18–25 somite embryos) only traces of the two-layered sort of epithelium are found; most of the gut lining is of a tall, irregular, pseudostratified nature. In grafts of pieces of primitive streak and head-process embryos, regions of epithelium typical of esophagus are encountered mostly as transitory zones between other types; in only one or two cases in all the grafts examined were small tubes found in which the major portion was composed of this epithelium. These traces appear to be found equally in grafts from various regions of the node field and anterior axis region of the blastoderm at the stages studied.

It is then evident that even the cells destined to form esophagus lining do not necessarily take on their peculiar arrangement in graft tubes; and that the conditions responsible for formation of this pattern in normal development are not duplicated in grafts either of the prospective esophagus region or of any other region of the early embryo.

3. *Respiratory-tract epithelium.*—The respiratory tract arises as the ventral complement to the esophagus, and, like the esophagus, is represented by a very small area in the

early blastodrem. One might therefore suppose that its differentiation in grafts would parallel the foregoing case and that only transitory traces of its epithelial pattern would be found. Actually, a great deal of epithelium of the irregular pseudostratified sort found in the main respiratory passages at 10 days' incubation occurs in grafts; and some of it is associated with cartilage and muscle elements, so that its identification as actual respiratory-tract epithelium seems justified. This epithelial type has been discussed previously (Rudnick, 1933); its irregularities and its tendency to grade into other epithelial types have also been noted. It is found not only in grafts of the post-branchial region of 18–25 somite embryos but also in those from the branchial region. It is also, as has been said, found in grafts from the node and anterior embryonic field of head-process and primitive streak blastoderms.

4. *Stomach epithelium.*—In the 10-day embryo the two divisions of the stomach contain characteristic epithelia, resembling each other, and probably representing a higher degree of differentiation than is found at any other level. Certainly the cellular pattern, especially pronounced in the ventricular region although characteristic also in the primary proventricular tube—the long, attenuated, regular cylindrical cells with nuclei crowded down to the base and with tufts of viscous secretion extending into the lumen from the distal border—represents a marked departure from the arrangement which would be assumed by homogeneous bodies in a tube growing under constraint.

Stomach epithelium of this differentiated sort, as well as the thickened pseudostratified, less specific stages which precede its formation, is found in grafts. It occurs in transplants of the closed or closing stomach region of 18–30 somite embryos, although the gut lining in such transplants by no means differentiates exclusively in this direction. A variant of the tall stomach epithelium in grafts is an epithelium equally tall, secreting in the same manner, but of uneven cell height and having the distal cell ends separated instead of being close packed and regular. This irregular stomach epithelium has been found continuous with the regular sort in grafts, as well as in combination with muscle layers, in both of which cases its identification seems certain. The same tall, irregular, epithelial type has been found composing vesicles that appear to be sensory-ectodermal rather than gut derivatives. An analogous tall, irregular epithelium is found in transplants of the closed branchial foregut. Although the specificity of such irregular epithelia is in doubt, it is worth noting that they as well as clearly identifiable stomach lining are found only in grafts from the node field in head-process stages and evidently are similarly restricted in the primitive streak blastoderm.

5. *Small intestine epithelium.*—By the 10-day stage, the small intestine lining has thinned from its original pseudostratified condition to a low columnar secreting epithelium with characteristic dark-staining nuclei (haematoxylin preparations). This sort of epithelium, next to the immature pseudostratified type, is probably the most frequent component of grafts from early stages. It may occur in transplants of the stomach and posterior regions from 18 to 30 somite embryos. In head-process and primitive streak blastoderms, it arises from all regions tested. In the head-process series it is clearly seen that although this low columnar epithelium may appear in grafts from any level, mesodermal relations characteristic of the small intestine are found only in grafts from the node field and posterior. Here we have, in addition to isolated epithelial areas, complexes comprising vesicles or tubes of low columnar secreting epithelium thrown into folds by local subepithelial aggregations of erythrocytes—sometimes very extensive—surrounded in turn by smooth muscle masses showing any degree of orientation or disorientation of

fibers. It is difficult not to interpret these structures as representing small intestine, and the folds as produced by the same sort of processes that create folds and ultimately villi in the small intestine. Transplants of the midgut of 18–30 somite embryos may show just such pictures. On the other hand, at least one clear-cut case has been examined where such a complex occurs in a graft containing no entoderm (402–1–2:ser. 2) originally. The source of the epithelial component in this case is in doubt; which, in turn, must make the origin of epithelium in such complexes in all other grafts a matter of speculation.

6. *Large intestine epithelium.*—The large intestine of the 10-day chick has an epithelium of strikingly characteristic appearance evidently involving no cellular specificity. In the anterior part of the tube the pseudostratified epithelium is a coherent layer with graded variations in height, so that a wavy border is produced. In certain regions of the tube tension must be a factor: the lining is separated into irregular blocks of tall cells, apparently pulled apart from neighboring groups, so that a deep scalloped effect results in section (Fig. 10). The posteriormost portion of the tube shows striking hypertrophy of the epithelium, the lumen being almost obliterated by strands of cells.

The wavy and scalloped epithelia are frequently encountered in grafts. The scalloped sort is found not only in transplants of the tailbud region in 18–30 somite embryos but also in grafts of the formed stomach (Figs. 9 and 12). In the head-process stage any level of the blastoderm is capable of forming pseudostratified epithelium which may locally show regularly graded cell height, or pronounced scalloping. Similarly, all regions are capable of forming hypertrophied knots of this epithelium which are identical in appearance with the occluded lumen of the rectum (Fig. 11). These structures are formed equally in the presence or in the absence of musculature. Again, it is only in the node field and posterior that such epithelial structures may be associated with other components of the gut tube. Two cases (6 and 7) described in the preceding section illustrate the perfection which may infrequently be attained in grafts of the node field. Usually the mesodermal matrix, when present at all, is lacking one of its components. It may consist only of a mesenchymal condensation, or only of muscle irregularly oriented with respect to the tube; if both components are present, their quantitative and spatial relations are very different from normal.

In this case as in that of the small intestine, then, the epithelium is a sort that may differentiate from any region of the blastoderm. The epithelial types are also found in grafts containing no entoderm at the time of transplantation. Organization factors combining these unspecific epithelia with mesodermal components to form structures corresponding—in the best cases almost exactly—to normal large intestine are present to a slight degree in the node field, and very strikingly present in the midstreak level of the head-process blastoderm; closer to the node in the definitive primitive streak stage. Such organization factors may operate in the absence of the entoderm, as is proved by one case (406–2–3:ser. 2) where a graft of the ectoderm and mesoderm of a primitive streak blastoderm shows a definitely recognizable gut tube suspended in a vesicle. The unspecific pseudostratified epithelium shows hypertrophy and almost occlusion characteristic of the rectal portion of the large intestine. Both submucosal and muscular layers are present; neither would ever be mistaken for the normal structure; but the tube is obviously a segment of gut, developed without entoderm's being utilized.

This survey of the differentiation of components of the gut in grafts would not be complete without the observation that the mesodermal portions as well as the epithelia may be found independently. Smooth muscle masses may occur without any accompanying

epithelium. Condensations of mesenchyme and muscle have been found surrounding tubes formed of epidermis. In a few cases rounded masses of smooth muscle covered with a flat membrane are found projecting into vesicles—structures suggesting intestine minus the epithelium. A series could be constructed linking such muscular nodules with fully developed intestine suspended in a similar situation, including cases where a minute epithelial sphere is located at the center of the muscular nodule and cases where a definitely intestinal tube is present, the epithelium of which still retains its immature pseudostratified character.

Throughout the foregoing section the fact has been emphasized that some quite characteristic gut structures are found in grafts of blastoderms from which the entodermal layer was removed before transplantation. The occurrence of even one such case would be a striking fact. Nevertheless, it should in justice be noted that throughout the series of grafts in question, in some of which the entoderm was completely removed and in some incompletely, there is a marked reduction in number and extent of structures referable to gut. Some grafts contain none at all; some merely epithelia which may locally show an arrangement characteristic of a certain gut region. A few cases contain both epithelial and mesodermal gut components; in none is a perfectly differentiated gut segment of definite level present. There is no well-formed graft of this series that contains more than a few traces of gutlike structures; whereas in a corresponding graft with entoderm intact, a large fraction of the total graft volume is composed of gut and gutlike complexes of all degrees of organization.

DISCUSSION

The foregoing section has been an attempt to describe and classify the configurations bearing some resemblance—close or remote—to the gut tube, as they occur in grafts. It must be clear that the classification of "differentiated gut" as against "epithelium accompanied by connective tissue, cartilage, or muscle" is a purely arbitrary one. Actually a closely graded series is present, ranging from gut tubes identical with normal ones, through distorted tubes retaining varying degrees of the normal arrangement of elements, down to vesicles and tubes completely or partially surrounded by slight muscle or mesenchymal condensations, and even to folded or tubular epithelia with no trace of surrounding mesenchymal material of graft origin. Of the first structures in the series there can be no doubt as to identity; of the last group of structures there can be only doubt.

It is possible that any epithelium of the blastoderm is capable of proliferating in the graft site to the extent of forming vesicles and sheets of unspecific pseudostratified cylindrical epithelium, as well as of the other types that have been described. Certainly other germ layers than entoderm can do so.[2] The mesenchyme of the host membrane evidently furnishes an excellent situation for growth of epithelium, and provides diverse relations permitting differentiation in various directions.

[2] Since the preparation of this manuscript, the valuable definitive publications of Hunt (1937*a* and *b*) have appeared. Hunt has followed the gut-forming areas from early stages, and shown clearly a correlation of this capacity with actual cellular migration from ectoderm to entoderm—if these terms are still permissible. The levels from which Hunt obtains gut-differentiation correspond very closely to those described here—in as far as comparable regions have been tested. Hunt prefers the interpretation that gut-differentiation in grafts is accomplished by cells which would form gut in normal development; in the present report, the regulative aspects of the case have been emphasized. It would seem that differentiation of organized gut from diffuse cells in the upper layers destined to form entoderm, but not yet actually in place, would require extensive regulation, and some supra-cellular organizing mechanism.

Epithelial pattern as found in the gut of the 10-day chick embryo is, then, capable of being reproduced in a random way in grafts of pieces of early blastoderms. As has been noted, these patterns depend morphologically on various arrangements of cells, and on cell function (secretion). These both are properties of epithelia in general; the inference is that there is nothing morphologically specific or unique about the entoderm or the gut epithelium in the embryonic period under consideration. It is only when the epithelium is organized with appropriate mesoderm to form gut tubes that specificity comes into the question; and this is a specificity of intercellular and tissue relations, not of intracellular structure. These rather obvious facts are understood in the following discussion of differentiation and prelocalization of the gut tube.

The regions of the gut showing approximately normal differentiation in grafts from early blastoderms are small and large intestine, and to a lesser degree (as regards frequency and perfection) stomach. Respiratory tract shows a differentiation that is characteristic, but not to be described as normal; its morphogenetic factors are not localized or operative in grafts until a relatively late stage (Rudnick, 1933). Esophagus and pharynx appear not to form as morphological units; nor do the cloaca and allantoic stalk.

This seriation with regard to perfection of organization has evidently no relation either to the antero-posterior sequence of appearance of organs, since its high point is in the midgut, or to the order of subsequent differentiation of various levels of the gut. A factor that must be of decisive importance is the mechanical complexity of the closure process, since it is most probable that normal-appearing gut in grafts must owe its origin to mechanics essentially identical with those of gut formation in the normally developing embryo. Evidently a simple gut tube closing directly from a flat splanchopleure, within a coelomic vesicle, is mechanically the most easily realized differentiation. Much less frequent—in any recognizable form—are gut regions complicated by secondary branching (i.e., proventriculus, lung), or where closure is associated with closure or growth of an accompanying ventral entodermal structure—esophagus, cloaca.

Whether the striking predominance of intestine among differentiated gut tubes is entirely a matter of differential expression in the graft environment—for mechanical or other reasons—or whether there is also something unique about the intestine field, in that it is very early organized with reference to the time when the primordium will actually appear, is a question that cannot now be answered. However, it is fortunate that at least one level of the gut can express its potencies in grafts and thus provide a basis for a sample localization scheme.

During its history the blastoderm seems always (as tested by grafting) to be a system equipotent or nearly so for the formation of gut tissues; but organized gut appears only in grafts from certain regions. Butler (1935) reports gut in grafts of all parts of the unincubated blastoderm; the only cases where she identifies gut of a definite level are in grafts from the posterior region—which corresponds to the primitive streak and node in potency and in fate. In the late primitive streak stage the present findings indicate an intestine-forming field just behind the pit, probably extending some distance posteriorly. In the head-process stage there is a striking shift of this field to a position definitely outside of and posterior to the node field, which is itself moving posteriorly at the same time. Only a very low percentage of cases of organized intestine occur in grafts from the node field. Potencies for forming organized stomach remain (as far as the data go—Cases 2 and 3, p. 383, restricted to the median portion of the node field, immediately around the pit.

The shift—whatever its nature turns out to be—of the intestine-forming field during the establishment of the head-process is comparable to the lateral shift in potencies for formation of thyroid and liver tissues, occurring during the same period. The result is that potencies become localized in the region which will, after suitable folding and cell migration, become the anlage in question. We have found no evidence that the localization process itself is to be explained on the basis of cellular migration, either in the entoderm or in the mesoderm.

The mesoderm—at least its splanchnic layer—is a factor that must always be taken into account in deciphering the capacities of the entoderm. The tendency of entodermal structures in grafts to be associated with the mesodermal tissues normally near them in origin, rather than to form coherent continuous entodermal systems, demonstrates that the entodermal germ layer is not necessarily a developmental unit, but is subordinated to regional organization. At present the indications are that, at least as late as the head-process and early somite stages, and probably much later, the entoderm is a system possessing a minimum of autonomy, both as to capacity for persistence, growth, and differentiation in experimental *milieux* and as to localization of potencies. The first point is illustrated by the fact that completely isolated entoderm has not been successfully grown in grafts (Hunt, 1934; Rawles, unpub.); only when some mesoderm is included are successful grafts obtained. Similarly, Waddington (1932) has observed that the entoderm will not undergo morphogenesis *in vitro*, although the upper germ layers will do so when separated from the entoderm. The second point is attested by the material reported in the present communication, where it is seen that no localization of epithelial type is manifest except where the entoderm is organized into units of gut tube—a process involving largely mesodermal differentiation—and that even under organized conditions the epithelial pattern is unstable. It is further seen that the entoderm itself is not essential to the formation of gutlike configurations in grafts.[3] Further evidence is seen in the case of liver and thyroid which differentiate as tissue masses tending very strongly to be associated with heart rather than with any organized gut (see Willier and Rawles, 1931; Rudnick, 1932; Dalton, 1935; Rawles, 1936).

The lack of autonomy displayed by the entoderm with regard to its own differentiation must somehow be reconciled with the profound early developmental effect of this layer on the overlying ones, in the process of formation of the primitive streak, as revealed by the experiments of Waddington (1933). The ability to direct primitive form-building movements may evidently be completely divorced from innate differentiation capacity.

SUMMARY

1. Early differentiation of the various levels of the gut tube, and of the histological elements of the gut, has been studied in chorio-allantoic grafts of the chick blastoderm.

2. Pieces of the primitive streak or head-process blastoderm may form essentially perfect segments of gut in the graft situation, as may transplants of the closed or closing gut from embryos of 14–30 somites.

3. Small and large intestine are the portions of the gut forming most frequently and most perfectly in grafts from primitive streak and head-process blastoderms. Distorted stomach is occasionally formed; pharynx, esophagus, respiratory tract, and cloaca are never found as organs.

[3] For that matter, Waddington (1932) has described other epithelia as taking the place of entoderm in entodermless embryos growing *in vitro*.

PLATE I

Photomicrographs of sections fixed in Bouin or Allen B-15, sectioned 7–8μ, stained in Heidenhain's iron haematoxylin.

FIG. 9.—Enlarged view of epithelium shown in Fig. 12. ×370

FIG. 10.—Epithelium of colon in 11-day normal chick. ×370

FIG. 11.—Epithelial knot in graft from median strip of a head-process blastoderm, anterior to the node, showing hypertrophy and cavitation typical of the occluded lumen of the rectum. ×132.

FIG. 12.—Section through grafted stomach region, 30-somite embryo, showing varieties of epithelium obtained (cf. Fig. 9). ×53.

FIG. 13.—Small intestine formed in graft of median region of level *E*, head-process blastoderm. Note perfect organogenesis, mesentery at upper left. ×132.

FIG. 14.—Section through gutlike complex in a head-process graft (same as Fig. 11) showing gradation of pseudostratified epithelium to regular columnar. ×132.

FIG. 15.—Section through small intestine, normal 10-day embryo (cf. Fig. 13). ×132

PLATE I

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4. In addition to perfectly formed or at least recognizable gut, epithelial tubes, vesicles, and sheets characteristic of all levels of the tract are found in transplants of pieces from the anterior embryonic and node levels of the blastoderm. These may or may not be accompanied by characteristic mesodermal structures referable to gut wall, and may or may not be derived from entoderm.

5. It is concluded that any epithelium of the embryo may take part in forming gut-like complexes in grafts, and that final localization of epithelial type has not taken place in the entoderm even after the closure of the gut.

6. In contrast to the lack of localization in the entodermal epithelium, the power of organ-formation of the midgut, as expressed by the occurrence of nearly perfect intestinal tubes in grafts, seems definitely localized in the blastoderm. In the head-process stage this intestine-forming field coincides with level *E* and the anterior median part of level *F* (Fig. 2); in the definitive primitive streak stage it is located immediately behind the node level.

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EXPERIMENTS ON SCALE OF ORGANIZATION IN EUPLANARIA DOROTOCEPHALA[1]

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FOR investigators in developmental physiology the parallelism between reconstitutional and embryological development should not go unnoticed. The similarities between the two processes are so numerous that one is led to the assumption that certain fundamental principles involved in these supposedly different kinds of development are identical. From the present state of knowledge it appears that such phenomena as physiological dominance, subordination, differential inhibition, differential tolerance, differential recovery, etc., are related to a dynamic differential or gradient which may be found either in a reconstituting piece of planarian tissue or in a developing echinoderm egg.

It has often been noted in this laboratory that short transverse pieces from the anterior region of the first zooid of *Euplanaria* may develop a perfectly normal head but lack entirely, or develop to only a limited degree, a posterior end or tail (Child, 1911*a*, 1911*b*, 1911*c*). These forms can aptly be compared to certain partial forms which develop from the animal portion of an echinoderm egg. It seems not unlikely that similar factors may underly these two different types of development. Since these tailless or partial forms in *Euplanaria* have so far been dealt with only incidentally and since a greater knowledge of their occurrence and possible modification is needed, a further analysis was undertaken.

MATERIAL AND METHODS

The material used in this investigation was the common flatworm of the Chicago vicinity, *Euplanaria dorotocephala*. In the majority of experiments animals 14–16 mm. in length, which had been collected several weeks previously and had been starved for approximately 2 weeks prior to section, were used. Since much work in this laboratory has shown that short pieces from the more posterior levels of the anterior zooid reconstitute largely into forms with strongly inhibited head types, only transverse pieces from the anterior levels were used in the present experiments. All sections were made by a single movement of a short sharp scalpel. The pieces were allowed to reconstitute in well water or in the test solutions in finger bowls or Erlenmeyer flasks at room temperatures. Parallel control experiments were always run if necessary. At the end of the reconstitutional period (approximately 2 weeks) the reconstituted pieces were observed under a microscope, and the type of reconstitution classified and recorded.

Animals classified as "tailless" had normal heads but developed no new tissue at the posterior end. The absence of new tissue was easy to determine by the fact that in the early stages new tissue has relatively less pigment than old. "Near-tailless" forms were stunted in appearance, possessing normal heads but only a small amount of new tissue at the posterior end. Other forms developed "normal tails" but had various head types

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which showed all degrees of inhibition from normal (I) through teratophthalmic (II), teratomorphic (III), anophthalmic (IV) to acephalic or headless (V).[2] In Tables 1–3 the percentages of the different head forms are given under the roman numerals I–V. Some pieces reconstituted into apolar forms which failed to give rise to either a head or a tail and had apparently lost all polarity. Bipolar forms, which reconstituted a head at both anterior and posterior ends, sometimes developed from short transverse pieces.

EXPERIMENTAL DATA

1. *The effects of size, level, and cut surface on frequency of tailless forms.*—In this series of experiments 100 pieces from similar animals were used in each of the experimental lots. The six different lots were sectioned as follows:

1. *A* pieces—anterior transverse pieces, one-sixteenth the length of the animal, from the region immediately posterior to the head
2. *B* pieces—pieces of the same length but immediately posterior to *A* one-sixteenth level
3. *A*+*B* pieces—anterior transverse pieces, one-eighth the length of the animal and comprising the same region as *A* and *B*
4. Head+*A* pieces—pieces including the head plus the anterior one-sixteenth of the animal
5. Head+*A*+*B* pieces—including the head plus the anterior one-eighth of the animal
6. Head pieces—made up of the head alone

The types of animals reconstituting from the pieces of these different lots are recorded in Table 1. Here it can be seen that *A* pieces reconstituted 36 per cent tailless, 23 per cent near-tailless, 32 per cent with normal tails, and 5 per cent apolar forms. Pieces from *B* level reconstituted 17 per cent tailless, 8 per cent near-tailless, 60 per cent with normal tails, and 5 per cent apolar forms. Of the *A* pieces, 4 per cent, and, of the *B* pieces, 10 per cent, died during the course of reconstitution. Pieces one-eighth the length of the animal (*A* + *B* pieces) reconstituted forms with 100 per cent normal tails. Pieces including the head plus *A* level gave rise to 3 per cent tailless, 5 per cent near-tailless and 92 per cent normal. Pieces made up of the head plus the anterior one-eighth (head + *A* + *B* pieces) reconstituted into 100 per cent normal animals. At the end of the reconstitutional period 80 per cent of the lot containing heads alone had died. Of the 20 per cent living, 17 per cent were tailless, while 3 per cent had developed a slight amount of new tissue at the cut surface.

The data clearly show that in short pieces of the same length the more active level (*A* pieces) reconstitutes more tailless and near-tailless forms than does a slightly less active level (*B* pieces). It also seems clear that the occurrence of such forms is dependent upon the length of the piece as well as the level (compare anterior eighths and sixteenths). The effect of cut surface is shown in those pieces with no anterior section (head attached), where the percentages of tailless and near-tailless forms are decreased. Heads alone prob-

[2] The different head types described and figured many times by Child and others are briefly: (I) *Normal*—head triangular, auricles at lateral margins, two distinct eyespots; (II) *Teratophthalmic*—head triangular, auricles in normal position, eyespots ranging from two complete structures connected by a narrow band of pigment to complete cyclopia; (III) *Teratomorphic*—head rounded, auricles anterior in position and approximated to the median plane up to a single median structure, single or apparently single median eyespot; (IV) *Anophthalmic*—head rounded and rudimentary, with or without a single median auricle, eyespots absent; (V) *Acephalic*—head completely absent. Lots of pieces reconstituting a high percentage of strongly inhibited head types are said to have a low head frequency, while those with a high percentage of normal or near-normal heads are said to have a high head frequency.

ably fail to reconstitute normal tails or live long because of an inability to dedifferentiate sufficiently.

2. *The effects of delay of anterior and posterior section on frequency of tailless forms.*—The object of these experiments was to test the effects of delay of anterior and posterior section on *A* sixteenth and *B* sixteenth pieces. In other words, an effort was made to determine whether or not there would be an increase or decrease in the percentage of partial (tailless and near-tailless) forms if the newly reconstituting head or tail was given as much as 24 hours' start in reconstitution; or to determine what effect, if any, an actively growing head or tail would have on the reconstitution of the opposing member.

As in the preceding experiments, six lots with 100 pieces per lot were used. In the first two lots (*A* pieces and *B* pieces) no delay in either anterior or posterior section was made. These lots, therefore, serve as controls for the following lots. In the third lot posterior section of *A* pieces was delayed 24 hours. In the fourth lot posterior section was

TABLE 1

THE EFFECT OF LENGTH AND LEVEL OF PIECE ON THE RECONSTITUTION OF PARTIAL FORMS IN *Euplanaria*

Section	Tailless	Near-Tailless	Apolar	Head Types of Forms with Normal Tails					Dead
				I	II	III	IV	V	
A (sixteenths)	36	23	5	30	.....	2	.....	.....	4
B (sixteenths)	17	8	5	44	2	2	3	9	10
A+*B*	.....	.....	.....	99	1	.....	.....	.....	.....
Head+*A*	3	5	.....	92	.....	.....	.....	.....	.....
Head+*A*+*B*	.....	.....	.....	100	.....	.....	.....	.....	.....
Heads	17	3	.....	.....	.....	.....	.....	.....	80

delayed for 24 hours in *B* pieces. In the fifth and sixth lots anterior section was delayed 24 hours in *A* and *B* pieces.

The data from these experiments are recorded in Table 2. It will be noted by comparing the results of these experiments with those recorded in Table 1 that *A* and *B* pieces from this stock reconstituted fewer partial forms. Since the animals used in this series were from a different stock than those used in the earlier experiments and since different stocks have been found to vary as regards head frequency (Rulon, 1936*a*), it was to be expected that the percentages of partial forms would not coincide exactly. When *A* pieces with 24 hours' posterior delay are compared with their controls (*A* pieces—no delay) it will be noted that the percentage of partial forms has been increased. Comparison of *B* pieces with 24 hours' posterior delay, however, shows little or no increase in the percentage of partial forms over controls. On considering the effects of 24 hours' delay of anterior section, it is found that *A* pieces differ but little from the controls, while *B* pieces show a slight decrease in frequency of partial forms. In both *A* and *B* pieces with anterior delay it will be noted that there is an increase in the percentage of inhibited head types in the animals reconstituting normal tails. This increase is particularly marked in the pieces from *B* level.

From these data it would appear that, given 24 hours' start, the head developing at

the anterior cut surface may inhibit a tail at the posterior cut surface in *A* pieces but not in *B* pieces. If the tail region be given 24 hours' start, there seems to be an inhibition in the formation of a head to some degree in *A* pieces and more so in *B* pieces. Delay of anterior section may likewise cause a slight decrease in the percentage of partial forms in *B* pieces while apparently not altering the percentage of such forms in *A* pieces.

3. *The effect of KCN on the frequency of tailless forms.*—Child (1911*c*, 1912, 1916) showed that certain inhibitory agents, such as KCN, lower the head frequency, that is, increase the frequency of forms with inhibited heads or no heads at all in short transverse anterior pieces of *Euplanaria*. It therefore seemed advisable in this work to test the effect of such an inhibiting agent on the reconstitution of tailless and near-tailless forms.

In these experiments four lots of 100 pieces per lot were used. Anterior and posterior sections were made at the same time. The first two lots (*A* and *B* pieces, one-sixteenth

TABLE 2

THE EFFECT OF DELAY OF ANTERIOR AND POSTERIOR SECTION ON THE RECONSTITUTION OF PARTIAL FORMS IN *Euplanaria*

Section	Tailless	Near-Tailless	Apolar	Head Types of Forms with Normal Tails					Dead
				I	II	III	IV	V	
A (sixteenths)—no delay.	20	6	.....	67	1	2	.....	.....	4
B (sixteenths)—no delay.	12	4	.....	60	3	4	2	5	10
A pieces—24-hr. posterior delay.....	33	20	.....	46	1	.....	.....	.....	.....
B pieces—24-hr. posterior delay.....	12	8	.....	78	1	.....	.....	.....	1
A pieces—24-hr. anterior delay.....	19	4	6	49	2	8	5	5	2
B pieces—24-hr. anterior delay.....	4	4	.....	38	1	4	4	39	6

the length of the intact animal) reconstituted in well water. The third and fourth lots from the same levels and of the same length reconstituted for the first 3 days after section in M/100,000 KCN and for the remainder of the experimental period in well water.

The results of these experiments are recorded in Table 3. It will be noted from these data that the controls (those pieces reconstituting for the entire period in well water) show a high percentage of tailless and near-tailless forms (50 per cent tailless and 20 per cent near-tailless in *A* pieces; 24 per cent tailless and 6 per cent near-tailless in *B* pieces). In pieces reconstituting for the first 3 days in KCN a somewhat different result is found. Pieces from the *A* level reconstitute 35 per cent tailless, 5 per cent near-tailless, and show an increase in the frequency of forms with normal tails. Pieces from the *B* level indicate a slight decrease in partial forms with 3 days' exposure to the test solution. It will likewise be noted that both levels show an increase in the percentage of inhibited head types in the test animals with normal tails. This is particularly marked in *B* pieces where 33 per cent of the reconstituted forms are acephalic as compared to the 1 per cent acephalic forms from the *B* level of the control.

These results seem to indicate that an inhibitory period immediately after section lowers the activity and thereby alters certain reconstitutional factors in short transverse pieces from anterior levels so that they tend to reconstitute into wholes or animals with normal tails and inhibited heads to a greater extent than in the controls.

TABLE 3

THE EFFECT OF M/100,000 KCN ON THE RECONSTITUTION OF PARTIAL FORMS IN *Euplanaria*

SECTION	TAILLESS	NEAR-TAILLESS	APOLAR	HEAD TYPES OF FORMS WITH NORMAL TAILS					BI-POLAR	DEAD
				I	II	III	IV	V		
A (sixteenths)—well-water control	50	20	1	20	.....	.....	.....	.....	.....	9
B (sixteenths)—well-water control	24	6	.....	48	5	1	1	1	1	13
A pieces—KCN sol. first 72 hr.	35	5	1	30	3	2	4	3	.....	17
B pieces—KCN sol. first 72 hr.	17	1	3	26	.....	.....	4	33	2	14

DISCUSSION

It has long been known that short transverse pieces of *Tubularia* will develop into certain partial forms (unipolar or bipolar partial hydranths) and that the production of such forms is dependent upon length of piece and level of the stem from which the piece was taken (Driesch, 1899; Morgan, 1901, 1902, 1903). The frequency of these partial forms has been found, in general, to be greater in pieces from the distal than the proximal regions. Also, pieces from the more proximal regions of the stem must be shorter than more distal pieces to produce partial forms.

Child (1926) in his work on *Corymorpha* found, with few exceptions, the same type of reconstitutional phenomena as observed by himself (1907) and earlier workers on *Tubularia*. He interprets the different types arising from pieces of the same size but from different levels in terms of the gradient conception. He points out that short pieces of a given length from a less active region may give rise to a complete individual or a complete bipolar hydranth, while a more active piece of the same length may develop only a hydranth, without a stem or base, or bipolar manubria—"because with the higher levels of activity the gradient is longer and the localization of parts takes place on a larger scale." Such differences in length or size in hydroids and other forms have been regarded by Child as resulting from differences in "scale of organization" determined by degree of dominance and dependent upon a dynamic physiological differential.

In *Euplanaria*, as in the hydroids, it has been shown that short pieces from different levels reconstitute differently and that this difference while quite distinct, can be altered in a number of ways. It has been shown in this work (Table 1) that the length of the pieces and the level from which they are taken determine to a large extent the type of reconstitution. Pieces from *A* sixteenth level reconstitute into a relatively high percentage of tailless and near-tailless forms (Tables 1, 2, and 3), while pieces of the same

length from *B* level, although giving rise to a number of such forms, do not reach the degree of taillessness found in *A* pieces. Pieces of a greater length (*A* + *B*) from these levels fail to reconstitute tailless forms.

It seems probable from the data that the situation in *Euplanaria* is to be interpreted much the same way that Child (1926) interprets his data on *Corymorpha*. In other words, short pieces from the anterior region are from a region of high activity, and, because of this activity, reorganization takes place on a larger scale. Since the size of the piece is limited, the only part of the individual forming is that part which is the most active in the complete animal.

It has been shown many times by Child and others that short transverse pieces from the more posterior regions of the anterior zooid of *Euplanaria* reconstitute into a high percentage of head-inhibited or headless forms. It has been noted, however, that a few hours' delay of posterior section of pieces from these levels will bring about the reconstitution of animals with 100 per cent normal heads (Child and Watanabe, 1935). Apparently the delay of posterior section permits the head-forming anterior region of the piece to become sufficiently activated to form a head regardless of certain inhibitory factors imposed when the posterior section is made. With a few hours' delay of anterior section, head frequency may be even more inhibited than in the controls, but, with longer delay, head frequency gradually increases and after 3 or 4 days' anterior delay the pieces may reconstitute 100 per cent normal heads. These experiments suggest that the inhibition of the head is related to factors arising at the posterior cut surface which reach their greatest intensity several hours after section and then gradually decrease.

In our experiments on the delay of posterior and anterior section of one-sixteenth pieces (Table 2) it was noted that 24 hours' posterior delay of *A* pieces brought about an increase in the percentage of tailless and near-tailless forms. The same delay with *B* pieces had little or no effect on the frequency of partial forms, but there was an increase in the head frequency of those forms with normal tails. These results would seem to indicate that, since *A* level is slightly more active than *B* level, the delay of posterior section permits a larger and possibly more active head to be initiated in the more active pieces, which results in a higher frequency of partial forms. With the slightly less active *B* level, posterior delay results in an increase in head frequency, but the activity apparently falls short of the threshold for the production of an increased number of partial forms.

With 24 hours' anterior delay in *A* level, the percentage of partial forms differs little if at all from the controls, but there is a slight decrease in head frequency of the animals with normal tails. With the same delay in *B* pieces, the percentage of partial forms as well as the head frequency of animals reconstituting normal tails is decreased. This lowering of head frequency with short-time anterior delay is in accord with the work of Child and Watanabe (1935). An explanation suggested by these data is that the 24 hours' anterior delay results in a slight inhibition of activity for head formation at the *A* level which finds expression only in those animals developing normal tails. In the *B* level, which is slightly less active than the *A* level, this inhibition of head-forming activity finds greater expression not only in lowering the head frequency of animals with normal tails but in decreasing the percentage of partial forms. This inhibition in head formation is undoubtedly related to conditions at the posterior cut surface which reach their greatest inhibitory action several hours after section and continue with decreasing effect during early stages of tail formation. The presence of such an inhibitory factor has been

discussed in some detail in previous work (Child and Watanabe, 1935; Rulon, 1936*a*, 1936*b*, 1937).

A large number of agents have been used in this laboratory by various workers (Child, 1911*c*, 1912, 1916, 1920; Behre, 1918; Buchanan, 1922; Hinrichs, 1924; Rulon, 1936*a*, 1937) to bring about an increase or decrease in the head frequency of animals reconstituted from short transverse pieces of *Euplanaria*. Child (1916) found that KCN was effective in lowering the head frequency in pieces from anterior levels and in increasing the head frequency of pieces from the posterior levels of the anterior zooid. These and other similar results together with the effects of delayed posterior and anterior section have been regarded as indicating that two antagonistic factors are concerned in determining head frequency: the activity of the cells directly involved in head formation and the activity resulting from posterior section which tends to inhibit head formation in pieces below a certain length. In the anterior pieces the direct inhibiting action of KCN on the head-forming cells overbalances its inhibiting effect on the posterior cut surface, and a decrease in head frequency results. In the posterior pieces the head-forming cells are less susceptible, and the effect of KCN in decreasing or blocking the activity of the posterior cut surface becomes evident, consequently head frequency increases.

Our experiments in which *A* and *B* pieces (one-sixteenth the length of the intact animal) were subjected for 72 hours to M/100,000 KCN (Table 3) indicate a lowering of physiological activity in anterior pieces with a subsequent decrease in the scale of organization in reconstitution. Pieces from *A* level treated with the KCN solution show a decrease in the percentage of tailless and near-tailless forms when compared to the well-water control. There is also a slight decrease in head frequency. Pieces from *B* levels, similarly treated, show a slight decrease in partial forms as well as a marked decrease in head frequency (33 per cent headless forms in test as compared to 1 per cent in the control). The only conclusion possible from these data seems to be that the KCN solution inhibited the active head-forming regions of the pieces.

In dealing with these partial forms, the question is likely to arise as to whether the tailless forms from short anterior pieces have the capacity to form tails at all. In other words, may they not lack tail-forming potency? Our experiments seem to show quite definitely that there is no such lack but that the occurrence of a tailless form is related to the activity of the piece. If this activity is altered, then the frequency of tailless forms is likewise altered. A large number of experiments (unpublished) have indicated that a few hours' delay of posterior section of pieces as long as one-eighth the length of the animal from the region of the fission plane may bring about the reconstitution of tailless forms. There can be no doubt that pieces from such a posterior region have the potency for tail formation even though it may not be expressed.

In conclusion, it may be pointed out that differences in scale of organization very similar to those in the reconstitution of short pieces in *Tubularia*, *Corymorpha*, and *Euplanaria* appear in the reconstitution of animal (apical) and vegetal (basal) halves or fourths of the early sea urchin blastula (Hörstadius, 1935). The animal portions usually develop into apical partial forms without entoderm or mesenchyme and with enlarged scale of organization of the apical region as compared with normal wholes. These partial forms, however, do not result from absence of potency for entoderm and mesenchyme but from a scale of organization too large for the piece. This is evident from the fact that, after temporary subjection to lithium, animal halves may develop entoderm and mesenchyme

and approach proportions of the normal whole (von Ubisch, 1925, 1929; Hörstadius, 1936).

Differential dye reduction in echinoderm development indicates a primary gradient in rate of reduction decreasing from the apical region basipetally and a secondary gradient opposite in direction, appearing before gastrulation and partially obliterating the primary (Child, 1936). In the isolated animal halves developing in normal environment this secondary gradient apparently does not arise, consequently the scale of organization determined by the primary gradient is larger than normal. After lithium treatment the primary dominance is decreased, the secondary gradient appears, and the scale of organization determined by the primary gradient is decreased. Apparently essentially similar factors are concerned in determining the apical partial forms in the sea urchin, the partial hydranths in *Tubularia* and *Corymorpha*, and the tailless forms in *Euplanaria*. Lithium decreases the scale of organization in the sea urchin just as KCN does in the short planarian pieces, and wholes, or forms approaching wholeness, result.

The development of vegetal halves and fourths of the sea urchin embryo is also comparable in certain respects to development of short planarian pieces with inhibited heads. In both sea urchin and planarian pieces there is apparently activation in the animal or anterior region of the piece. In the sea urchin vegetal piece, particularly in the vegetal fourth, the scale of organization determined by the secondary gradient is disproportionately large because the primary gradient and dominance are largely absent. In the planarian piece the activation resulting from posterior section retards or inhibits head development. In both cases two opposed gradient factors are concerned. In the development of partial hydranths with large scale of organization from pieces of the more distal levels of *Tubularia* and *Corymorpha* stems and of whole individuals from pieces of the same length from lower levels similar gradient relations are evidently concerned.

SUMMARY

1. With *E. dorotocephala* (14–16 mm., approximately 2 weeks without food preceding experiment) as material, pieces one-sixteenth the length of the intact animal from *A* level reconstitute a higher percentage of tailless and near-tailless forms than do pieces from *B* level. Pieces one-eighth the length of the intact animal from the same regions fail to reconstitute such partial forms. The reconstitution of animals with inhibited tails is, therefore, dependent upon the length of the piece, the level from which it was taken, and, since different stocks vary as regards percentages of reconstitution of such forms, general physiological condition of the animals.

2. Delay of posterior section for 24 hours causes an increase in tailless and near-tailless forms in *A* but not in *B* pieces. Delay of anterior section for 24 hours causes a slight decrease in tailless forms in *B* but not in *A* pieces. Delay of posterior section increases the head frequency of animals with normal tails, while 24 hours' anterior delay lowers the head frequency in similar forms.

3. An initial exposure of 72 hours to M/100,000 KCN lowers the percentage of tailless and near-tailless forms as well as the head frequency in animals reconstituting from *A* and *B* levels.

4. Attention is called to certain similarities in the reconstitution of short planarian and hydroid pieces and of isolated animal and vegetal pieces of the sea urchin embryo.

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RESPONSES OF THE BARNACLE TO SOME STRONG ELECTROLYTES AND TO UREA, GLUCOSE, AND GLYCEROL[1]

(One figure)

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PREVIOUS studies on the responses of various animals to mineral and aliphatic acids, sodium salts of mineral and fatty acids, and to alcohols have demonstrated several types of quantitative correlations between intensity of stimulation and the specific nature of the ions and molecules concerned (see Allison and Cole, 1933–34, and papers quoted therein; Sizer, 1935–36). New data have been secured from the barnacle on the sodium and potassium salts of the mineral acids and on urea, glucose, and glycerol, which have allowed: (1) comparison of stimulating effects of the salts with those of their corresponding acids; (2) description of two opposite types of responses, opening and closure, the occurrence of which depends upon the concentration of the substances used; and (3) determination that equally stimulating solutions of the strong electrolytes and of the nonelectrolytes have markedly different osmotic pressures.

MATERIALS AND METHODS

Under normal conditions in natural sea water the cirri of the barnacle (*Balanus balanoides*) execute remarkably constant rhythmic movements and are extremely sensitive to various environmental stimuli, including changes in the chemical content of the sea water (Cole, 1932). In populations of about one hundred individuals or more under experimental conditions described elsewhere (Cole, 1931–32), from 50 to 85 per cent of the animals will be found regularly active and will continue so until stimulated in some way. It is believed that percentages less than 50 indicate the effect of chemical stimuli naturally occurring in the sea water (Allison and Cole, 1935).

During three successive summers[2] six different populations averaging ninety-three individuals each have been tested by two different observers (W. H. C. and J. B. A.), and the data have been combined. The procedure was as follows. After the animals were observed to be normal in behavior, the number of regularly active animals was recorded. The running sea water was then turned off, and the experimental solution containing the substance to be tested was substituted at the same temperature ($17 \pm 0.2^{\circ}$ C.) and rate of flow (250 ± 25 cc. per min.). At successive minute-intervals the number of regularly active animals was recorded until no further change took place (a maximum of 10 min.). When the number of open animals was plotted against minutes, three types of responses were found as follows: (1) it remained constant (nonstimulating concentrations); (2) it increased to a constant value; or (3) it decreased to a constant value, either (*a*) gradually or (*b*) irregularly by first reaching a minimum value and then increasing to the constant value. The latter method seems to involve a "recovery" or "adaptation" of certain in-

[1] Aided by a grant (to W. H. C.) from the National Research Council, 1934.

[2] All the observations were made at the Mount Desert Island Biological Laboratory during the summers of 1932–34.

dividuals and will be mentioned later in this report. All solutions were made up in from 6 to 8 liters of sea water. In no case was the pH of sea water (8.1±02.) as determined colorimetrically changed significantly by the added substance. Tests were also made with thirteen binary mixtures of the three sodium salts, urea, and glucose.

RESULTS

Besides the closing effect, which is a well-known response of the barnacle to many sorts of stimuli (Cole, 1932), it was unexpectedly found that each one of the substances tested, if used at concentrations below those which cause closure, would cause opening. (Glycerol was not studied for the opening effect.) There are thus two concentrations for each substance which are nonstimulating; between those concentrations closed animals are stimulated to open and to remain regularly active; above the higher critical concentration open animals are stimulated to close. This double effect bears a resemblance to the common accelerative and depressor effects of certain "drugs" and related substances. Furthermore, for the sodium salts, glucose, urea, and glycerol, a recovery or adaptation effect was found similar to that reported previously for some of the sodium salts of the fatty acids[3] (Cole, 1931–32). This indicates that some animals which are at first stimulated to close become adapted to the stimulating substance and reopen. For $NaNO_3$ at all concentrations above the closure threshold, recovery was very marked, amounting sometimes to nearly 40 per cent; for Na_2SO_4 it was noticeable only at two of the higher concentrations (0.12 and 0.13 N); and for NaCl it was indicated only at three of the lower concentrations (0.07, 0.08 and 0.09 N). For urea at seven of the higher concentrations (0.08–0.14 M) recovery was marked (about one-third that for $NaNO_3$), but for glucose at three of the higher concentrations (0.11–0.13 M) it was just noticeable.

The experiments with potassium salts and with glycerol were carried only far enough to demonstrate qualitatively the similarities of their effects to the other substances. Concentrations of the potassium salts necessary to produce the same effects were found to be about one-fifth those of the corresponding sodium salts, and the range of concentrations from the threshold for opening to the maximum value for closure was much shorter than for the sodium salts. In the glycerol experiments two different supplies were used (Merck's Reagent and Baker's c.p. analyzed), giving data which were roughly similar but consistently different in magnitude. Both samples contained unknown amounts of fatty acids and esters as impurities, which are highly effective stimuli for the barnacle, and which are probably responsible for the variable results.

For the salts, NaCl, Na_2SO_4, $NaNO_3$, and the nonelectrolytes, urea and glucose, sufficient data were secured over a period of three years to be analyzed quantitatively. The average percentages of open animals at the end of 4 minutes' exposure to the solutions are given in Table 1. The average percentage of open animals in sea water was 62, so that values above 62 indicate the opening effect, and values below 62 indicate the closing effect. The percentages for urea and glucose at each concentration were so nearly alike that they were averaged. To illustrate effects of the salts, the NaCl data are plotted in the upper half of Figure 1. In the lower half are plotted the averages for urea and glucose. When the data for each substance are plotted separately, it is found that all the curves are similar, differing only quantitatively. This similarity in shape suggests that the same groups of receptors are involved in stimulation by all the substances. The sym-

[3] The recovery effect was very marked with Na heptylate and clearly noticeable with valerate after the end of the first minute. It was not noticed with the other fatty-acid salts used.

metry of the curves suggests that only one group of receptors is involved in the process of closure and that another group is involved in the opening process.[4] For the salts the opening part of the curve is distinctly skewed toward the lower concentrations; for the nonelectrolytes the opening effect was not studied at enough concentrations; so that the dotted line indicates only predicted values. Another feature that appears from the plots is that the nonelectrolytes with concentrations expressed in molarities are approximately equal in stimulating effectiveness to the sodium salts with concentrations expressed in normalities, in spite of the great difference in the osmotic pressures of their solutions.[5]

TABLE 1*

AVERAGE PERCENTAGES OF ANIMALS OPEN IN SIX POPULATIONS AT THE END OF 4 MINUTES' EXPOSURE TO THE SOLUTIONS LISTED

Concentration (Added to Sea Water)	NaCl	Na_2SO_4	$NaNO_3$	Urea and Glucose
0.00	62.0	62.0	62.0	62.0
0.01	64.4	63.0	63.5	.....
0.02	65.0	62.6	65.6	.....
0.03	67.1	65.6	69.7	.....
0.04	66.6	67.4	67.3	.....
0.05	62.7	67.5	65.0	.....
0.06	55.5	63.1	64.2	68.4
0.07	48.8	56.5	55.1	64.1
0.08	42.0	48.1	49.7	56.5
0.09	31.3	35.2	41.8	52.8
0.10	19.5	30.0	27.2	43.8
0.11	7.6	28.8	19.2	41.3
0.12	5.6	13.6	17.7	32.9
0.13	3.6	18.9	11.8	23.3
0.14	2.5	2.5	9.6	13.1
0.15	1.9	3.2	5.3	11.6
0.16	.....	.....	.....	11.8
0.18	.....	.....	.....	8.0
0.20	.....	.....	.....	3.9

* Average percentage open in sea water was 62. Concentrations of the salts are expressed in normality and of the nonelectrolytes in molarity. Temp. = 17 ± 0.2° C.; rate of flow of solutions = 250 ± 25 cc. per min.; volume of container = 625 cc.; average volume of barnacle population and of stone seat = 400 cc.

In Table 2 are displayed the data from binary mixtures of the salts and nonelectrolytes which cause closure. For every mixture tested, the effect is plainly additive and may be expressed in terms of a concentration of one of the components necessary to give the same response.[6] Calculated values of the percentage of open animals are figured on

[4] It was previously found that closure initiated by the mineral and fatty acids appears to involve the presence of three or more receptor groups. The opening effect could not be produced by those acids (Cole and Allison, 1932–33).

[5] The osmotic pressures of equally effective concentrations were calculated from the freezing-point depressions determined experimentally.

[6] The thresholds for closure varied somewhat from day to day. Before testing the mixtures, the thresholds for each of the components were experimentally determined in order to calculate the equally effective solutions of each component and the percentage opened expected from the mixture. Some of the values in the fourth column of Table 2, therefore, differ slightly from those interpolated from the average curves in Fig. 1.

the assumption that the higher critical concentration (closure threshold) for each substance contributes an equal amount of available energy, since an equal amount of work

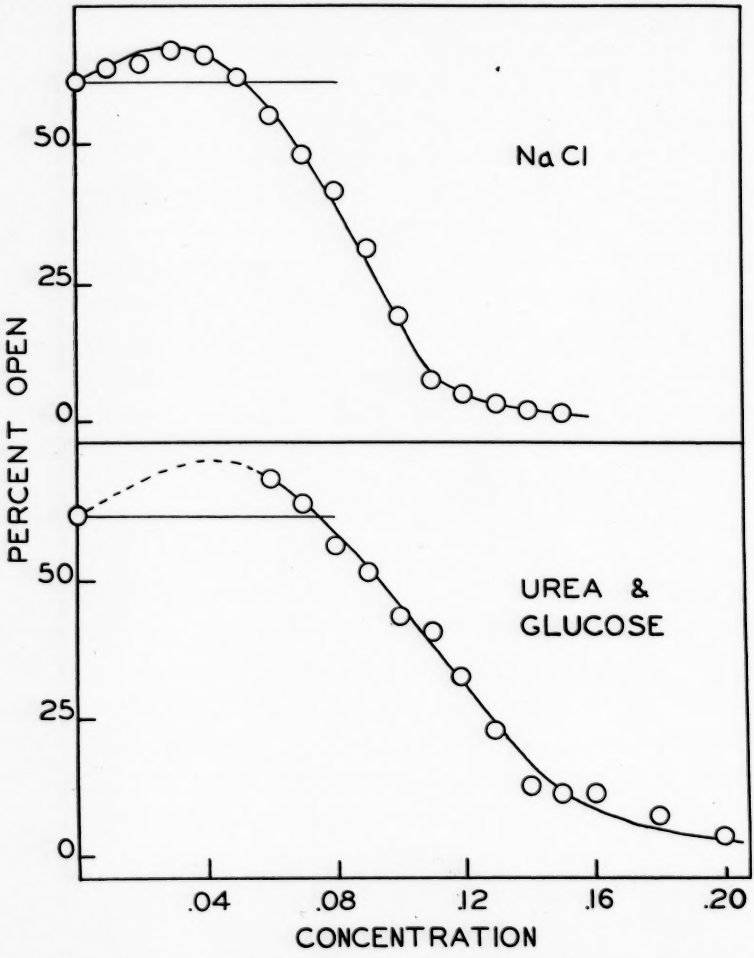

FIG. 1.—The average percentages of open animals in six populations of barnacles after 4 minutes' exposure to the solutions are plotted as ordinates against normal concentrations of NaCl and molar concentrations of urea and glucose as abscissas. The average percentage of open animals in pure sea water is indicated by the fine line drawn through ordinate 62 parallel to the concentration axis. Points above that line indicate opening, and points below it indicate closure. The dotted part of the lower plot represents predicted values for opening.

is done by it. For example, when the closure threshold of NaCl (0.05 N) is mixed with a subthreshold concentration, such as 0.02 M glucose, the percentage of open animals ob-

tained is almost identical to that from 0.095 M glucose solution. This concentration of glucose may be calculated by adding the higher critical concentration of glucose (0.075 M) to the concentration of glucose in the mixture. This additive effect supports the conclusion that identical groups of receptors are involved in stimulation to close by these substances.

TABLE 2*

THE PERCENTAGE OF OPEN BARNACLES OBTAINED FROM BINARY MIXTURES AT THE END OF 4 MINUTES

Mixture	Equivalent to Single Solution	Percentage Open	
		Experiment	Calculated
$NaNO_3$ and NaCl: N N			
0.04 0.05	0.10 $NaNO_3$	38	31
$NaNO_3$ and Na_2SO_4:			
0.06 0.02	0.08 Na_2SO_4	46	46
0.06 0.06	0.12 Na_2SO_4	14	15
NaCl and urea: N M			
0.05 0.04	0.115 urea	36	37
0.05 0.07	0.145 urea	20	18
0.05 0.14	0.215 urea	2	1
NaCl and glucose:			
0.05 0.02	0.095 glucose	49	51
0.05 0.07	0.145 glucose	17	18
0.05 0.08	0.155 glucose	5	10
$NaNO_3$ and urea:			
0.04 0.07	0.10 $NaNO_3$	40	35
0.08 0.07	0.14 $NaNO_3$	9	8
Urea and glucose: M M			
0.07 0.07	0.14 urea or glucose	14	14
0.11 0.07	0.18 urea or glucose	4	7

* The threshold concentrations for closure are as follows: NaCl = 0.051 N; Na_2SO_4 = 0.062 N; $NaNO_3$ = 0.063 N; urea and glucose = 0.075 M.

The five substances may be listed in the following order of effectiveness for closure: NaCl > 1/2 Na_2SO_4 > $NaNO_3$ > urea = glucose. This order of salts is the same as that found for the corresponding mineral acids (Cole and Allison, 1932–33). There is little difference between the three salts, especially between 1/2 Na_2SO_4 and $NaNO_3$, but the slight specificity of the anion is real and shows itself also in recovery. As indicated above, recovery is most marked from $NaNO_3$, much less marked from Na_2SO_4 and NaCl. The animals show least recovery from the most effective stimulating substance, so that the order of recovery is just reversed to the order of stimulation and differs from it quantitatively.

DISCUSSION

It has been suggested that in each barnacle there are two groups of receptors involved in stimulation by salts and nonelectrolytes; one group mediating the opening response, the other closure. Whatever the mechanism of the reaction, the net effect measurable is probably a summation of two activities, one producing opening and the other closure. It is not often that the compound nature of a biological process is so strikingly revealed and so easily measured. From the plots in Figure 1 it can be assumed that the ascending arm of the opening curve and the lower part of the closure curve are determined almost solely by the receptors for opening and for closure, respectively. The intermediate parts of the curves, however, must be determined by summations of the opening and the closure effects. It is planned to present in a later report a more detailed analysis of this compound response.

The opening effect is not produced by the acids. That must mean either that the receptors for opening are not stimulated by the acids or, more likely, that the threshold for opening is never reached because stimulation of the receptors for closure occurs at very low hydrogen ion concentrations, thus preventing effective stimulation of the opening receptors.

The shapes of the curves as well as the experiments on mixtures prove that the sodium salts, the potassium salts, urea, glucose, and glycerol all act in essentially the same way, whichever type of receptor is primarily involved. The similar effectiveness of urea and glucose can be correlated with the fact that their particles (molecules) bring to the receptor interfaces similar amounts of kinetic energy without any interference from specific attributes of the particles. According to that assumption the effectiveness of any chemically stimulating substance could be correlated with the net change in kinetic energy at the receptor interface. The pressure produced by bombarding particles against the interface may, however, be modified providing the particles are electrically charged, as ions are. For instance, the effectiveness of each kind of dissociated salt tested shows a correlation with primary valence forces, i.e., with the magnitude of the charge on the particles (ions). Thus any general attempt to account for the pressure at receptor interfaces produced by electrolytes must take into consideration electrostatic forces as well as the kinetic energy of their solutions. The sodium salts, therefore, should be less effective than would be predicted from the osmotic pressures of their solutions because a certain amount of kinetic energy borne by them has become bound energy due to electrostatic forces. In other words, the effective concentration of the ions is less than the actual concentration at the interface. The potassium salts would be considered more effective than the sodium salts because of the greater dynamic characteristics of the potassium ion as contrasted to the sodium ion.

Specific attributes of stimulating substances are often correlated with dynamic characteristics such as particle weight, mobility, adsorbability, etc. For instance, Hopkins (1932) reported for the oyster that stimulating efficiency could be approximately correlated with the product of ionic mobility and ionic weight. The order of magnitude of that product coincided with the order of stimulating efficiency of certain salts. Thus, as also in the barnacle, KCl was a more effective stimulating agent than sodium chloride. But sodium nitrate was a better stimulating agent for the oyster than sodium chloride, just the reverse to the order found for the barnacle. Obviously urea and glucose could not be included in such a method of calculation. In fact, any attempt to correlate effectiveness of electrolytes and nonelectrolytes must take into consideration at least three

factors concerned wlth the environment and with the receptor interface: osmotic pressure, electrical-field strength, and a force associated with the specific nature of each particle. The effect of an environmental stimulus upon a receptor will depend upon the physical and chemical characteristics of the receptor interface as well as of the environment itself. The development of these concepts further at the present time would be largely speculative, since so little is known concerning the physical chemistry of the receptor-environment system.

SUMMARY

1. Using percentages open of a population of barnacles, *B. balanoides*, as criteria of stimulation by various substances the following order of effectiveness has been found: $KNO_3 > KCl > \frac{1}{2}K_2SO_4 > > > > > NaCl > \frac{1}{2}Na_2SO_4 > NaNO_3 >$ urea = glucose > glycerol.

2. Excepting glycerol, which was not tested for the opening effect, each substance shows two contrasted effects. At the lower concentrations the number of open and regularly active animals increases, while at the higher concentrations closure occurs.

3. Recovery from or adaptation to stimulation by all the substances except the potassium salts occurs in different amounts. The order of recovery is $NaNO_3 >$ urea > glucose $= \frac{1}{2}Na_2SO_4 > NaCl$.

4. The data lead to the suggestion that there is only one group of receptors involved in the process of closure, with another group involved in the opening process.

5. Binary mixtures of the substances used for closure show additive effects which support the suggestion that only one group of receptors is involved in closure.

6. Molar concentrations of urea and glucose are nearly equal in effectiveness to the corresponding equivalent concentrations of the sodium salts, indicating that mere increase in osmotic pressure of the receptor-environment system is not the only factor in stimulation by these electrolytes and nonelectrolytes.

7. Theoretical considerations are presented as a basis for future interpretation of the results.

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STUDIES ON THE FIREFLY. II. THE SIGNAL SYSTEM AND COLOR VISION IN PHOTINUS PYRALIS[1]

(One figure)

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A. The Signal System in *Photinus pyralis*

INTRODUCTION

THE mating habits of the firefly, *Photinus pyralis*, according to McDermott (1911) and Mast (1912), are essentially as follows: At dusk the male and female emerge from the grass. The male flies about, approximately 50 cm. above the ground, and emits a single short flash at regular intervals. The female climbs some slight eminence, such as a blade of grass, and perches there. She ordinarily does not fly at all, and she never flashes spontaneously, as does the male, but only in response to a flash of light, such as that produced by the male. If a male flashes within a radius of three or four meters of a female she usually responds, after a short interval, by flashing. "The male then turns directly toward her in his course and soon glows again. Following this the female again responds by glowing and the male again apparently takes his bearings, turns and directs his course toward her" (Mast, 1912, p. 260). This exchange of signals is repeated, usually not more than five or ten times, until the male reaches the female and mates with her.

Mast (1912) observed that males "seldom if ever" respond to the flashes of other males, and he therefore concluded that they somehow distinguish between the flashes produced by the female and those produced by other males. He listed (1912, p. 265) the following possible factors involved in this:

> (1) The female is always at rest when she glows while the male is ordinarily on the wing; (2) the form of the luminous area differs in the two sexes; (3) there is always a certain time relation between the glow of the male and that of the female in response to it; (4) the quality of the light produced by the two sexes may differ; (5) the intensity may differ; (6) the duration of the glow may differ.

Mast maintains that he eliminated all these factors "save possible difference in the duration of the glow."

The question as to how the male distinguishes between the flashes of male and female was thus left unsettled. The present investigation was made in an attempt to answer this question. The first step taken was to measure accurately the time relations in the flashing signals.

[1] The writer wishes to express his gratitude to Professor S. O. Mast for kindly interest in the work and generous assistance in the preparation of the manuscript; to H. S. Barber, of the United States National Museum, for identifying the organism investigated; and to Master Samuel Damon for faithful assistance in the field.

EXPERIMENTAL

1. *The interval of time between successive flashes of males in flight.*—The intervals between successive flashes of males were measured as follows: A specimen flying about near by and flashing periodically was selected and closely observed. A stop watch was started each time the male flashed, and simultaneously a second stop watch was stopped, read, and reset. The results were dictated to an assistant, who recorded them. This was repeated with many individuals under various conditions, and in observations on some individuals as many as seventy consecutive intervals were measured. The results obtained are presented in Table 1.

TABLE 1*

THE INTERVAL OF TIME BETWEEN SUCCESSIVE FLASHES IN FREE FLYING MALES

Date (1933)	Temp. (C.)	Number of Individuals Studied	Total Number of Measurements	Mean Interval between Successive Flashes (Sec.)	Average Deviation from Mean (Total for All Individuals) (Sec.)	Per Cent Deviation from Mean (Total for All Individuals)
7/13	21.7	9	45	7.77	.85	10.90
7/9	22.8	4	33	6.00	.46	7.67
6/23	23.3	16	124	6.21	.37	5.97
6/24	23.3	12	177	6.10	.52	8.53
7/10	23.3	10	116	5.75	.42	7.30
7/6	26.1	7	60	5.67	.72	12.70
6/22	26.7	26	104	4.91	.42	8.57
7/20	26.7	11	63	5.21	.42	8.10
7/22	28.3	10	46	4.44	.41	9.20
General averages and totals	24.7	105	768	5.78	.51	8.77

* Each horizontal row represents the mean results for one evening.

Table 1 shows that on a given evening the intervals between successive flashes in a given male deviated, on the average, 8.77 per cent from the mean interval for the evening; that the mean interval of all those measured was about 5.8 seconds; that 90 per cent of the measurements fell within the limits 4.2–7.8 seconds, inclusive; and that the mean intervals on the different evenings differed considerably from one another, depending on the temperature.

The relation between temperature and frequency of flashing is shown graphically in the upper curve in Figure 1. This curve shows that the interval between flashes decreases as the temperature increases. The temperature coefficient (Q_{10}) from 23° to 29° C. is approximately 1.7, which value is in fair agreement with that (1.9) obtained by Snyder and Snyder (1920) in less extensive observations on an unidentified species.

2. *The interval of time between the flash of the male and the flash of the female in response to it.*—As noted by McDermott (1911) and Mast (1912), the female of *P. pyralis* responds readily to an artificial light under proper conditions, as well as to the flash of a male. The interval between the stimulus (flash of male or flashlight) and the response of the female is of the nature of a reaction time and will be called the "response interval" of

the female. The response intervals of twenty-three females responding to flashes produced by males, or by a flashlight were measured with a stop watch. The results obtained are presented in Table 2. This table shows that the response intervals of a given female varied, on the average, 5.67 per cent from their mean; that the mean response interval for all the measurements was 2.06 seconds; that 90 per cent of the measurements fell within the range 1.4–2.8 seconds, inclusive; and that the mean response interval differed somewhat in different females, depending on temperature.

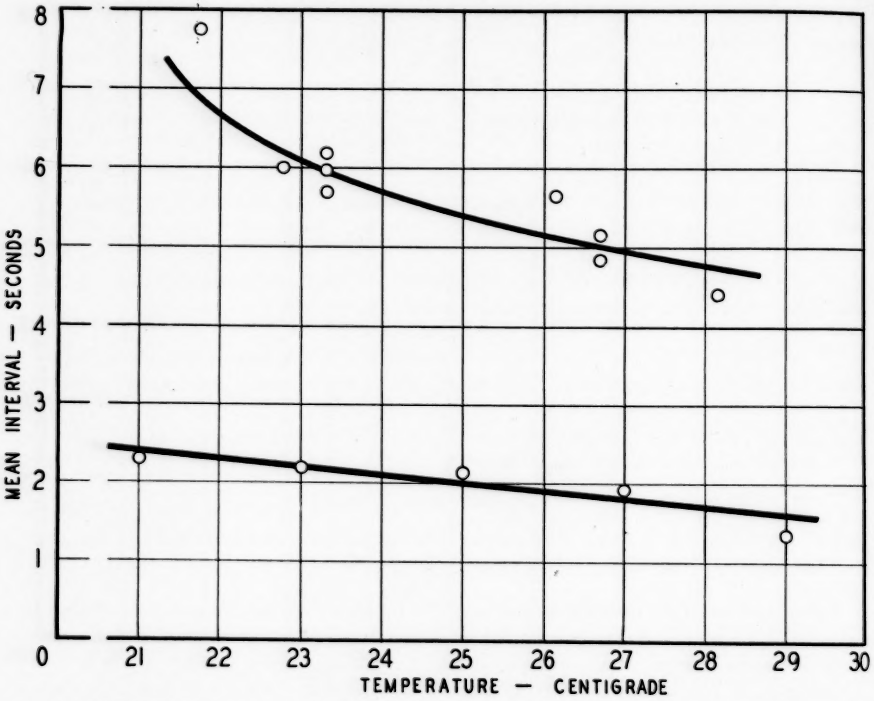

FIG. 1.—Relation between temperature and flashing in the firefly. Upper curve, each point represents the average interval between successive flashes of males on one evening, plotted against the temperature which obtained on that evening (data from Table 1); lower curve, each point represents the average length of the interval between the flashes of males, or of a flashlight, and the responses of females, over a range of 2° (data from Table 2).

The relation between response interval and temperature is shown in the lower curve in Figure 1. This curve shows that the length of the response interval of the female decreases with increase in temperature. The temperature coefficient (Q_{10}) for the response interval is approximately 1.5.

By comparing Tables 1 and 2 it will be seen that although the per cent deviations of the measurements of the response interval of the female are only slightly smaller than those of the measurements of the interval between successive flashes in the male, the

actual deviations are much smaller. The female, in fact, regulates her response interval with truly remarkable accuracy.

3. *Stimulation of the male by artificial light.*—The fact that the female invariably responds to the flash of the male after an interval of approximately 2 seconds suggested that the male might respond to an artificial light if it were flashed, like that of the female,

TABLE 2*

THE INTERVAL OF TIME BETWEEN THE FLASHES OF MALES OR OF A FLASHLIGHT AND THE FLASHES OF FEMALES IN RESPONSE

Date	Temp. (C.)	Number of Measurements on Same Female	Maximum Interval (Sec.)	Minimum Interval (Sec.)	Mean Interval (Sec.)	Average from Mean Deviation (Sec.)	Per Cent Deviation from Mean
7/27/33	21.0	20	2.7	1.9	2.17	.15	6.92
7/13/34	21.0	7	2.4	2.1	2.22	.09	4.23
7/13/34	21.0	16	2.5	2.1	2.32	.09	3.88
7/7/33	21.0	9	2.8	2.3	2.55	.17	6.67
7/5/33	22.3	10	2.8	2.7	2.78	.04	1.44
7/28/33	22.3	36	2.6	2.0	2.34	.12	5.13
7/18/33	23.0	27	2.6	1.9	2.31	.13	5.63
7/18/34	23.0	24	2.4	1.7	1.92	.13	6.77
7/16/34	24.0	28	2.7	2.2	2.37	.10	4.22
6/29/33	24.0	29	2.0	1.4	1.59	.12	7.56
7/18/33	24.4	22	2.7	2.1	2.38	.13	5.47
6/28/33	25.5	22	2.6	1.7	2.10	.16	7.62
7/29/33	25.5	40	2.1	1.2	1.51	.13	8.61
7/14/34	25.8	7	2.3	1.8	2.10	.12	5.72
7/14/34	25.8	13	2.4	1.9	2.21	.12	5.36
7/15/34	25.8	34	2.4	2.0	2.24	.10	4.39
7/19/34	26.0	9	2.5	1.6	2.16	.19	8.80
7/17/34	26.0	16	2.6	2.0	2.32	.12	5.20
7/19/34	26.0	31	2.4	2.0	2.16	.10	4.63
7/20/34	27.0	17	2.3	1.9	2.11	.12	5.68
7/20/34	27.0	9	2.0	1.6	1.76	.09	5.12
7/20/34	27.0	21	2.1	1.6	1.88	.11	5.86
7/30/33	29.4	33	1.6	1.1	1.35	.08	5.93
Averages and totals	24.5	480	2.4	1.8	2.06	.11	5.69

* Data divided into five groups, according to temperature, as averaged in plotting the lower curve in Figure 1.

2 seconds after his flash.[2] This experiment was performed by resting the end of a five-cell flashlight on the ground so that the light was directed into the grass at an angle,[3] and

[2] McDermott (1911) apparently used a similar system, flashing after "three to five" seconds, but he did not investigate the signals.

[3] Using this powerful flashlight, it was found that directing the light straight at the male did not usually elicit a response unless he was a long distance away, whereas he readily responded at any intermediate distance if not exposed to the direct rays of the flashlight. This shows that the male will not respond to light above a certain intensity. It was found, in addition, that a light intensity to which males responded

flashing it about 2 seconds after each flash of a male flying near by. The male turned and flew toward the flashlight in precisely the same manner that males respond to the flash of a female. The method was astonishingly successful, and it enabled the writer to exert an almost magical control over the male firefly. Individuals were "attracted" straight to the flashlight from distances of over 50 feet without difficulty, and fifteen or twenty males were often "attracted" to the flashlight at once (Buck, 1935).

4. *Orientation.*—A number of males were etherized, and, in each, one eye was completely painted over with opaque black enamel. It was found that these males gave no response whatever to flashes from a flashlight located on the blinded side, whereas they responded accurately to the same light when stimulated on the side of the functional eye. In the latter instance they flew toward the light sideward, keeping the functional eye oriented in the direction of motion. This experiment shows, first, that the response is certainly a visual one and not dependent on any sensitive tissue other than the eye, and, second, that orientation to light in the firefly is clearly not in accord with Loeb's theory of orientation in insects, which demands equal action of the light on symmetrically located receptors.

5. *The signal system.*—It was found that the male responds equally well to the flashlight whether the duration of the flash is 1 or $\frac{1}{10}$ second (estimated). This fact shows that Mast's conjecture that the male probably recognizes the flash of the female by reason of its being slightly different in duration from that of the male is not tenable.

It was found, in addition, that the male responds to the flashlight whether the area of the source of light is 1 square millimeter or 1 square decimeter; whether the flashlight is above, below, or to the side of the male, or moving either horizontally or vertically; whether the intensity of the light from the flashlight is very low or many times that of the light emitted by the female;[4] whether the color of the light is green, yellow, orange, or red.[5] These results confirm the conclusions of Mast that no possible difference in the color, intensity, relative motion of the light, or difference in the form of the luminous organ is of any importance in the discrimination between flashes produced by male and female. It was found, however, that males do not respond to flashes of artificial light under any circumstances unless they occur about 2 seconds after the flashes produced by the males. This strongly indicates that the response of the male depends upon the interval between his flash and that of the female.

Further evidence in support of this conclusion was obtained as follows: Males were "attracted" (one at a time) from some distance, and then, after they had approached to within less than 1 m. of the flashlight, the flashes were varied in diverse ways and the effects upon the responses of the males noted. The results obtained are summarized as follows: (1) two or three flashes in very rapid succession 2 seconds after the flash of the male—normal response; (2) flash at the same time as the flash of the male—no response;

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early in the evening often was too intense for use late in the evening. This indicates that what the male responds to is not so much the absolute intensity of the light as it is the difference between its intensity and that of the environmental illumination.

[4] From the average intensity of the light emitted by *P. pyralis* (1/100 candle; Coblentz, 1912) and the fact that males and females respond to one another at all distances between 1 cm. and 3 m., it is seen that in nature these fireflies respond to flashes of any illumination between 1/900 and 100 m.c., a relatively wide range. The precise limits of this range were not ascertained, but it was found in a previous study (Buck, 1937) that flashing is inhibited by intensities above about 210 m.c.

[5] Color vision will be treated in detail in sec. B.

(3) flash 1 or less seconds after the flash of the male—no response; (4) flash between 1.5 and 2.5 seconds after the flash of the male—usually response, depending somewhat on temperature; (5) flash 3 or more seconds after the flash of the male—no response.

As a crucial experiment a male was captured, held between thumb and forefinger, and gently pinched at intervals so that he emitted a flash approximately 2 seconds after each flash of a free flying male. The free male turned and flew to the captive male in precisely the same manner that males respond to a female or to an artificial light. This was repeated many times with different males, and the imitation of the female was so accurate that often the free male, after he had signaled with and approached the male held in the fingers, attempted to mate.

These results demonstrate conclusively that if there is any difference between the light emitted by the two sexes it has nothing to do with the ability of the male to distinguish between the flashes of the female and those of other males. They also make it practically certain that the discrimination by the male is dependent upon the regularity of the response interval of the female.

B. Color Vision in *Photinus pyralis*

INTRODUCTION

The term "color vision" is used in this paper in the sense commonly employed in insect physiology—meaning responses elicited by visual stimulation by lights of diverse wave-lengths. Bertholf (1931) and others have pointed out that the term is inapplicable in a strict sense to insects, because we do not know whether the subjective sensations of "color" are the same as in man.

The earlier literature on color vision in insects has been well reviewed by Graber (1883 and 1884) and Gross (1913), and the more recent by Imms (1931), Bertholf (1931), Eltringham (1933), and Mast (1936). The results presented show that in most insects color vision ranges roughly from 3500 to 6000 A, with the extreme limits extending perhaps as far as 2350 A in the ultraviolet and as far as 6770 A in the red.

METHODS

In the following experiments a flashlight and a series of "Wratten" light filters were used. In each test one of the filters was placed over the lens of the flashlight and covered light-tight with black paper in which a small hole was made in front of the filter.

In testing color vision in the female, a flash of the colored light transmitted by one of the filters was directed at her and the result noted. If she did not respond, the intensity of the light was changed by moving the flashlight nearer or farther away from her, or by using a more or a less intense source of light. If, in each of five consecutive tests, the female responded by flashing about 2 seconds after the flash of colored light, it was concluded that she saw the light.

In testing the male, a specimen on the wing was selected and illuminated by a flash of light transmitted by one of the filters, 2 seconds after each of his flashes. If the male responded to this treatment by turning toward the flashlight and flying to within 15 cm. of it from a distance of at least 3 m. it was concluded that he saw the light transmitted by the filter being tested.

Each filter was tested with many individuals of both sexes.

RESULTS

A summary of the results obtained is presented in Table 3. This table indicates that color vision in both sexes extends from some point between 5200 and 5600 A (in the green) to at least 6900 A (in the deep red), the latter being the longest wave-length yet recorded as visible to any insect.

The results obtained are perfectly trustworthy in the instances in which responses were obtained, that is, it is certain that color vision extends at least from 5600 to 6900 A (and almost certainly into the infrared, since the transmission of filter No. 88 is only 5 per cent at 7000 A), but the nonresponses are subject to some question because, as pointed out by Bertholf (1931), it is never possible to be certain that a nonresponse is due to the limit of vision's having been reached, or merely to the light's having been too weak.

TABLE 3*

RESPONSE OF *Photinus pyralis* TO LIGHT OF DIFFERENT WAVE-LENGTHS

"Wratten" Filter Number	Color	Range of Transmission in Visible Spectrum (Ångstroms)	Per Cent Transmission at Maximum Wave-Length in Visible Spectrum	Response
75	Blue-green	4700–5200	19 at 4900	No
58	Green	4800–6100	60 at 5200	Yes
74	Green	5100–5600	15 at 5300	Yes
22	Orange	5600–7000+	83 after 6400	Yes
72	Amber	5900–7000+	11.5 at 7000	Yes
29	Red	6100–7000+	69 after 6900	Yes
88	Infrared	6900–7000+	5 at 7000	Yes

* In the transmissions of the filters, values of less than 1 per cent are neglected. The results were the same in male and female.

The reason for believing that the lower limit of color vision lies somewhere above 5200 A is that whereas the fireflies responded readily to light transmitted by filter No. 58 at distances of 2 or 3 m., they did not respond to light transmitted by filter No. 75, even at a distance of 10 cm., representing a nine-hundred-fold increase in intensity. Calculation from the percentage transmissions of filter Nos. 58 and 75[6] and from the spectral energy emission data for the flashlight lamp used[7] shows that the total energy transmitted by filter No. 75 is about 12 per cent of that transmitted by No. 58. Nevertheless, since No. 75 proved ineffective even at intensities nine hundred times those which are effective with filter No. 58, it is concluded either that color vision does not extend as low as 5200 A or that the sensitivity of the eye of the firefly in the range of wave-lengths transmitted by filter No. 75 is less than one-one-hundredth of its value in the range transmitted by filter No. 58.

[6] *Wratten Light Filters* (10th ed.; Eastman Kodak Co., 1929). Pp. 86.

[7] The flashlight lamp used was Westinghouse No. 605, 13.9 lumens per watt, 6.0 volts, 0.5 amp., color temp. 2680° K. The author is indebted to Dr. C. S. Woodside, of the Westinghouse Laboratories, for the emission data.

SUMMARY

1. The male of the firefly *Photinus pyralis* flashes at fairly regular intervals, averaging 5.8 seconds. The length of the interval varies inversely with temperature.

2. The female of *P. pyralis* responds to the flash of the male by flashing approximately 2.1 seconds after his flash. The length of this response interval varies inversely with temperature.

3. The male is able to distinguish between flashes produced by the female and those produced by other males.

4. The male responds to flashes of artificial light and to flashes produced by other males if they occur about 2 seconds after his flash. This shows that no possible difference in the color, duration, or intensity of the light produced by the two sexes, or difference in the relative motion or form of the luminous area, is of any importance in enabling the male to distinguish between flashes emitted by male and female.

5. The essential factor in the ability of the male to distinguish between flashes produced by the female and those produced by other males is the fact that the female invariably responds to the flash of the male at a nearly constant interval of 2 seconds.

6. Color vision in both sexes of *P. pyralis* extends from some point between 5200 and 5600 A (in the green) at least as far as 6900 A (in the deep red), the latter being the longest wave-length of light yet recorded as being visible to any insect.

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SOCIAL MODIFICATION OF THE ACTIVITY OF ANTS IN NEST-BUILDING

(Three figures)

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SOCIAL behavior of various groups of animals has been studied by many naturalists, and important contributions have already been made to our knowledge about social behavior, especially of the social insects. Recently, some biologists have attempted to study the general social phenomena in the animal world and have started various lines of study. One of these is the study of animal aggregations, started by Allee (1931). This line of work is concerned with the physiological effects of crowding upon the individuals composing the crowd. Through the work of Allee and his followers many interesting facts concerning the effects of crowding have already been discovered.

Another line of the study of general social phenomena is that of population growth started by Pearl (1925). This line of work is concerned with the experimental studies of the growth of animal population in comparison with that of human population.

The third line of study of general social phenomena of animals has been independently carried on by Deegener (1918), Alverdes (1925), Picard (1933), and others. These authors have done a great deal in collecting the isolated facts, scattered in numerous special publications, about the social life of various groups of animals and in organizing them into comprehensive synthetic treatises.

The work which I am going to report in a series of papers, of which the present paper is the first, is concerned with experimental studies of some general social phenomena in ants. The special social activities of ants, or activities which are peculiar to ants, such as division of labor, care of young, common activities in defense and attack, slavery, relation with aphids and other insects, etc., have already been extensively and intensively studied by numerous entomologists, especially Wheeler (1910, 1923), Forel (1928), Wasmann (1915), Escherich (1917), and Brun (1924). In spite of the richness of the contributions which the myrmecologists and entomologists have made to our knowledge about the special social activities of ants, there has been little experimental work from the standpoint of general sociology.

My experimental study of general social phenomena in ants was started in the spring of 1936 by a series of analytic and synthetic studies of their nest-building activities. The results obtained are reported in two separate papers, of which the present one is concerned with the modification by social environment of the nest-building activities of individual ants.

MATERIAL AND METHODS

The common black ant, *Camponotus japonicus* var. *aterrimus* Emery, was used for all experiments reported in the present paper. A colony of this variety of ants is usually composed of a few wingless females, a few or many winged females and males, and thousands of workers. The workers vary greatly in size and may be classified, according to size, into at least three classes: the small, medium-sized, and large workers. The

average body length of the small workers is about 9 mm.; that of the medium-sized workers and large workers, about 11.5 mm. and 15 mm.

Camponotus japonicus var. *aterrimus* Emery is widely distributed in China, and its occurrence has been recorded in the following provinces: Hopei, Shantung, Hunan, Kiangsu, Chekiang, and Fukien (Wheeler, 1930). This variety of ants was selected for the following reasons: (1) The large size allows their activities to be observed easily and clearly. (2) Polymorphism in the size of workers allows individual ants to be easily distinguished or recognized without marking them with coloring matter or other materials, which often produce injurious effects. (3) On account of the wide distribution and abundance of this variety of ants, they are easily collected.

Workers of various size were used for experimental studies, and sexual forms were excluded because (1) since the primary object of the present study is to find out the general relations between the individuals in a society, it is better to eliminate the disturbing factor of sex by using a group of animals without sexual difference; (2) the sexual forms of ants ordinarily do not take part in the nest-building activities of the colony.

The ants were collected on the campus or in the vicinity of the National Tsing Hua University. Collections were made frequently, at least once a week, in order to insure a constant supply of fresh material for study. They were generally collected a few days before the beginning of an experiment. Only in exceptional cases, when experiments were continued for several weeks, were ants used after a month or more in the laboratory.

In order to avoid injurious effects of environmental conditions in the ordinary laboratory rooms, the experiments were carried out in a building situated in the Goldfish Garden of the University, where the conditions are nearly similar to the natural environmental conditions. In this building there is a spacious room which is well ventilated, evenly lighted, and yet not exposed to direct sunlight. The temperature in this room varied from 25° C. to 32° C. during the period in which these experiments were carried on.

The ants collected from the field were kept in large glass containers, supplied with a piece of wet sponge or a little moist earth, covered with cheesecloth, and put in a dark cool place. Ants belonging to different colonies were kept in separate containers. They were occasionally fed with honey or with sugar water on a small piece of sponge. At the beginning of each experiment the ants to be used were carefully examined in order to avoid use of individuals which were accidentally injured in collecting and handling them.

NEST-BUILDING ACTIVITIES OF ANTS IN ISOLATION

The nest-building activities of ants have long been well known to naturalists, and the different types of ant nests have already been studied and described in detail (Wheeler, 1910; Forel, 1928). But this activity as a general phenomenon of co-operative social work has not yet been investigated. My own studies along this line have been first analytic, then synthetic.

The analytical part of the work consists of a study of the nest-building activity of individual ants in isolation. Normally, nest-building is a social activity and individual ants work as members of a group. But we cannot conclude from the ability of nest-building by a group that all ants can also build a nest when in isolation. Experiment is needed to settle this question.

The experiment planned for studying nest-building in isolation was started on May

19; 70 workers were used. Each worker was introduced into a separate working bottle, shown in Figure 1, which was made of a half-pint milk bottle covered by a piece of cheesecloth fastened to the mouth of the bottle by a rubber band. As nest-building material, about 130 cc. of air-dried sandy soil, which had been finely pulverized and passed through a sieve, was put into each bottle. This was followed by 35 cc. of water, which soaked into the soil and made it moist enough for nest-building. The surface of the moist earth was made smooth by sprinkling over it at first a little very finely pulverized dry soil and afterward a fine spray of water from a washing bottle such as used in the chemical laboratories. The 70 working bottles thus made were not only similar in size and form but also in the volume and the moisture content of the contained earth.

Fig. 1.—Working bottle

The 70 workers used for this experiment were not selected with reference to size; they included large, medium-sized, and small workers. They were introduced into the 70 working bottles, one ant into each bottle. Each ant was then in a new world entirely isolated from the others. After a few minutes in this isolated condition, some ants started the work of nest-building. Four hours after introduction into the bottle, 47 of the 70 workers had already started excavation of nests. Twenty-three hours after being introduced into the bottle, 62 of the 70 ants had started the work of nest-building. Of the remaining 8 workers which had not yet started, 6 had begun work $21\frac{1}{2}$ hours later, and the other two had begun work 70 hours after introduction. Hence, within 70 hours all the 70 ants had started nest-building.

This experiment shows that all workers are capable of nest-building in isolation but that this ability is not identical among the individual ants. Some ants, in response to the nest-building stimuli (absence of old nests, presence of moist earth, etc.) in the new environment of isolation, began building very early; others began later or very late.

The isolated ants differ not only in the time of beginning the building but also in many other ways as regards details of construction. Most ants selected a spot near to the source of the light, i.e., the southern window of the room, and against the glass wall of the bottle for beginning work. Others started building in other places in the bottle. Most ants worked consistently in one spot; but a few dug two or more shallow cavities in different places in the bottle instead of concentrating their energy in constructing one deep nest. Some ants worked continuously and persistently until the nests or holes which they made were one or more inches deep and a considerable amount of earth which they dug out of the nests had accumulated around the openings. Others worked intermittently and dug out only a little earth to deposit around the openings of the shallow cavities.

NEST-BUILDING ACTIVITIES OF ANTS IN ASSOCIATION

The synthetic part of this study, i.e., the study of the nest-building activities of ants in association, will be reported in the following: On June 3, I selected from a stock of *Camponotus* 36 sound and healthy workers—12 large, 12 medium-sized, and 12 small

workers. The large workers varied in body length from 14.1 mm. to 16.6 mm., averaging 15.03 mm.; the medium-sized, from 10.5 mm. to 12.2 mm., averaging 11.23 mm.; the small, from 8.2 mm. to 9.8 mm., averaging 9.15 mm. The body lengths of these ants were measured at the end of this experiment by means of a caliper, after the ants were etherized.

Each of these 36 ants was distinguished by a letter, "L," "M," or "S," and a number, from 1 to 12. The letter "L" meant large; "M," medium-sized; "S," small worker. The numbers from 1 to 12 were the numbers of the individuals belonging to each size-class.

These 36 ants were kept in isolation or in association in two kinds of bottles: the working bottles and the resting bottles. The working bottles with moist earth in them were prepared according to the method described in the previous section. In these bottles the ants carried on their work of nest-building. In order to prevent the ants from excessive nest-building work, no moist earth was supplied; rather, there was in each resting bottle a piece of wet sponge for supplying moisture. Occasionally, the ants were fed with dilute sugar water while they were in the resting bottles; the frequency of feeding and the concentration of sugar water was identical for all the 36 ants.

During the period of experimentation the ants were transferred from the resting bottles into the working bottles for a period of 6 hours every day, after which they were transferred back into the resting bottles.

The experiments with the 36 ants lasted 12 days. During the first three days, the ants were kept isolated in 36 resting bottles and were transferred into 36 working bottles, one in each bottle, to work in isolation for 6 hours daily. In the second 3 days, the 36 ants were combined into 18 pairs. Each of these 18 pairs was kept in a separate resting bottle during the resting hours and was introduced into a separate working bottle, where the ants were allowed to work in association for 6 hours every day. During the third 3 days, they were recombined 3 by 3 into 12 groups, and these 3-ant groups were kept in 12 resting bottles and allowed to work for 6 hours daily in 12 working bottles. In the last 3 days, they were separated again and kept isolated both during the working and during the resting hours.

Concerning the daily work of each ant or group of ants, the following records were taken: (1) the reaction time and (2) the weight of earth excavated. The reaction time is the time elapsed between the entrance of ants into the working bottle and the beginning of their building activity. This interval of time was recorded in number of minutes. In case the ant did not start to work in the 6-hour working period, it was recorded as "360+." The weight of earth excavated is the amount of the earth which the ants brought up in the form of pellets from the nest and deposited around the entrance of the nest. While preparing the working bottles, special attention was paid to making the surface of the earth smooth and fairly compact so that the pellets deposited on it could be brushed away. At the end of the 6-hour working period, all the pellets accumulated on the surface of the earth were carefully brushed together and transferred into vials. After having been air-dried in the vials, the collected masses of pellets were weighed. The weight of these masses of pellets was taken to represent the amount of work done by the ant, or the ants, within the 6 working hours.

The data obtained from the experiments described above are recorded in Tables 1–4. M10 and S3 died within the period of experimentation; hence their data are not included in the tables. From the data in these tables the following conclusions were derived.

TABLE 1

REACTION TIME OF ANTS WORKING IN ISOLATION AND IN GROUPS*

Ants	In Isolation (June 4–6)		In Groups of 2 (June 8–10)		In Groups of 3 (June 11–13)		In Isolation (June 14–16)	
	M.R.T.	Dev.	M.R.T.	Dev.	M.R.T.	Dev.	M.R.T.	Dev.
L1	182+	8	22	3	25	1	360+	159
L2	130+	60	10	15	12	12	6	195
L3	360+	170	30	5	13	11	360+	159
L4	142+	48	10	15	27	3	142+	59
L5	49	141	35	10	2	22	360+	159
L6	360+	170	4	21	4	20	2	199
L7	247+	57	13	12	11	13	240+	39
L8	244+	54	4	21	3	21	124+	77
L9	141+	49	45	20	20	4	265+	64
L10	147+	43	8	17	131	107	241+	40
L11	156+	34	8	17	19	5	74	127
L12	124+	66	108	83	24	0	242+	41
Av.	190+	75	25	20	24	18	201+	110
M1	212+	9	17	1	12	30	271+	144
M2	230+	27	2	14	3	39	298+	171
M3	207+	4	57	41	125+	83	62	65
M4	225	22	8	8	20	22	126+	1
M5	360+	157	30	14	2	40	121+	6
M6	5	198	14	2	5	37	2	125
M7	259+	56	8	8	3	39	6	121
M8	248+	45	12	4	257+	215	360+	233
M9	26	177	7	9	4	38	127+	0
M11	298	95	1	15	15	27	6	121
M12	158+	45	20	4	21	21	20	107
Av.	203+	76	16	11	42+	54	127+	99
S1	7	175	4	40	14	18	19	134
S2	96	86	5	39	19	13	242+	89
S4	57	125	13	31	20	12	243+	90
S5	360+	178	22	22	20	12	244+	91
S6	338+	156	24	20	122+	90	13	140
S7	175+	7	1	43	2	30	136+	17
S8	63	119	127+	83	3	29	8	145
S9	244+	62	157+	113	12	20	173	20
S10	228	46	8	36	98	66	74	79
S11	300+	118	10	34	17	15	282+	129
S12	133+	49	108	64	21	11	248+	95
Av.	182+	102	44+	48	32+	29	153+	94
A.A.	192+	84	28+	26	33+	34	160+	101

* M.R.T., mean reaction time; Dev., deviation; Av., average; A.A., average of three averages.

TABLE 2

WEIGHT OF EARTH EXCAVATED BY 1 ANT WORKING IN ISOLATION*

Ants	Weight of Earth Excavated in Isolation (In Milligrams per 6 Hours of Work)		
	Averages of Three Trials of First Period of Isolation (June 4–6)	Averages of Three Trials of Second Period of Isolation (June 14–16)	Averages of Both Periods of Isolation
L1	627	0	314
L2	356	553	455
L3	0	0	0
L4	61	243	152
L5	676	0	338
L6	0	146	73
L7	43	58	51
L8	195	90	143
L9	32	75	54
L10	85	95	90
L11	162	655	409
L12	278	23	151
Av.	210	162	186
M1	90	11	51
M2	446	11	229
M3	165	168	167
M4	191	239	215
M5	0	21	11
M6	1,023	657	840
M7	86	957	522
M8	147	0	74
M9	434	104	269
M11	67	496	282
M12	166	197	182
Av.	256	260	258
S1	671	663	667
S2	521	18	270
S4	111	18	65
S5	0	148	74
S6	30	61	46
S7	592	57	325
S8	39	52	46
S9	45	56	51
S10	87	177	132
S11	115	23	69
S12	335	98	217
Av.	231	125	178
A.A.	232	182	207

* Av., average; A.A., average of three averages.

1. *The reaction time was shorter when the ants worked in association than when they worked in isolation.*—Table 1 shows that when the ants worked in 2-ant or 3-ant groups the mean reaction time was, on the average, 28+ minutes or 33+ minutes; when they worked in isolation the mean reaction time was 192+ minutes or 160+ minutes.

2. *The amount of accomplished work was more when the ants worked in association than when they worked in isolation.*—When isolated, each ant excavated, on the average, 0.207 gm. of earth in 6 hours (Table 2). When associated, the amount of excavated earth was greatly increased; it was 3.649 times more when the ants worked in 2-ant groups,

TABLE 3

WEIGHT OF EARTH EXCAVATED BY 2 ANTS WORKING IN ISOLATION OR IN GROUPS*

Ants	Weight of Earth Excavated by 2 Ants (In Grams per 6 Hours of Work)		
	When Working in Isolation (1)	When Working in Groups (2)	Ratio (2):(1)
L1, M1	0.365	1.555	4.260:1
L2, M2	0.684	1.920	2.807:1
L3, M3	0.167	0.893	5.347:1
L4, M4	0.367	2.646	7.210:1
L5, M5	0.349	2.529	7.246:1
L6, M6	0.913	1.682	1.842:1
L7, S7	0.376	1.329	3.535:1
L8, S8	0.189	0.829	4.386:1
L9, S9	0.105	0.335	3.190:1
L10, S10	0.222	1.694	7.631:1
L11, S11	0.478	1.200	2.510:1
L12, S12	0.368	1.380	3.750:1
M7, S1	1.189	2.492	2.096:1
M8, S2	0.344	1.652	4.802:1
M11, S5	0.356	1.331	3.739:1
M12, S6	0.228	1.002	4.395:1
Av.	0.419	1.529	3.649:1

* Data in column (1) were obtained by adding the data of individual ants in Table 2.

(Table 3) and 3.456 times more when they worked in 3-ant groups (Table 4) than when they worked in isolation.

3. *The associated ants worked with greater uniformity than the isolated ants.*—Based upon the data of the mean reaction time, average deviations had been calculated and recorded in Table 1. These deviations are much smaller (26 and 34) in ants working in groups, and larger (84 and 101) in ants working in isolation, showing greater uniformity in the former than in the latter.

4. *The accelerating effect of association was greater for the slow workers than for the rapid workers.*—Table 5 shows the mean reaction times of the three best workers (M6, S1, S8) and three poorest workers (L3, M8, S5) of the 36 ants investigated. It is clear from

these data that there may be some increase in the mean reaction time by working in association with other ants in the case of the best workers, but there is a great reduction in the case of the poor workers. Thus, the mean reaction time of the best worker (M6) is 3.5 minutes in isolation, but 14 minutes in the 2-ant group and 5 minutes in the 3-ant group. In this case the reaction time is increased. The poorest worker (L_3) did not work at all during the 6 hours in isolation. But, when associated with other ants, it started to work within 30 or 13 minutes after introduction into the working bottle.

TABLE 4

WEIGHT OF EARTH EXCAVATED BY 3 ANTS WORKING IN ISOLATION OR IN GROUPS*

Ants	Weight of Earth Excavated by 3 Ants (In Grams per 6 Hours of Work)		
	When Working in Isolation (1)	When Working in Groups (2)	Ratio (2):(1)
L_1, M_1, S_1.....	1.032	2.134	2.068:1
L_2, M_2, S_2.....	0.954	3.375	3.538:1
L_4, M_4, S_4.....	0.432	2.715	6.285:1
L_5, M_5, S_5.....	0.423	3.810	9.007:1
L_6, M_6, S_6.....	0.959	1.123	1.171:1
L_7, M_7, S_7.....	0.898	3.499	3.896:1
L_8, M_8, S_8.....	0.263	0.708	2.692:1
L_9, M_9, S_9.....	0.374	1.700	4.545:1
L_{11}, M_{11}, S_{11}.....	0.760	2.085	2.743:1
L_{12}, M_{12}, S_{12}.....	0.550	1.826	3.320:1
Av.....	0.665	2.298	3.456:1

* Data in column (1) were obtained by adding the data of individual ants in Table 2.

DIFFERENCE BETWEEN THE EFFECT OF A RAPID CO-WORKER AND THAT OF A SLOW CO-WORKER ON THE WORK OF AN INDIVIDUAL

The experiments described in the preceding section proved that the activity of an individual is greatly modified by its social environment. The experiments to be reported in the present section were planned to find out the difference between the effect of a rapid co-worker and that of a slow co-worker on the work of an individual. These experiments were carried out as follows:

From a healthy colony of *Camponotus*, I selected 3 workers with different rates of work and of slightly different size so that they could be easily distinguished. One, a rapid worker, was designated "R_1"; another, a slow worker, "S_1"; a third, whose rate of work was medium, "M_1." These ants were selected in the morning. As soon as selected, M_1 and R_1 were transferred together into one resting bottle, in which they were allowed to remain for a sufficient length of time to become accustomed to each other; and S_1 was transferred into another resting bottle.

In the afternoon of the same day, I transferred M_1 and R_1 from the resting bottle into a working bottle, in which they were allowed to work for 2 hours. During this

period, with the help of assistants, I took the following records of work: (1) the reaction time of M1 as measured by a stop watch; (2) the reaction time of R1; (3) the number of pellets of earth which M1 had carried from the interior of the nest to the outside in every 5-minute interval from the time of starting the work to the end of the 2-hour working period; (4) the same record as (3), but for R1. At the end of the 2-hour working period, R1 was discarded and M1 was introduced into the resting bottle of S1, in which they were allowed to remain together overnight. The next morning M1 and S1 were transferred together into a working bottle. They worked in this bottle for 2 hours, and their work was recorded in the same way as that of M1 and R1. The results of the experiment are presented in Table 6 and graphed in Figure 2. They show clearly that the

TABLE 5

DATA SHOWING THAT THE ACCELERATING EFFECT OF ASSOCIATION IS GREATER FOR SLOW WORKERS THAN FOR RAPID WORKERS

Ants	Mean Reaction Time in Minutes						Lengthening (+) or Shortening (−) of Reaction Time by Working in Groups
	When Working in Isolation			When Working in Groups			
	June 4–6	June 14–16	Average	In 2-Ant Groups June 8–10	In 3-Ant Groups June 11–13	Average	
Rapid workers:							
M6.....	5	2	3.5	14	5	9.5	(+) 6
S1.....	7	19	13	4	14	9	(−) 4
S8.....	63	8	35.5	127+	3	65+	(+) 29.5+
Slow workers:							
L3.....	360+	360+	360+	30	13	21.5	(−) 338.5+
M8.....	248+	360+	304+	12	257+	134.5+	(−) 169.5±
S5.....	360+	244+	302+	22	20	21	(−) 281+

work of M1 was differently modified by its co-workers. With a rapid co-worker, the work of M1 was also more rapid; with a slow co-worker, the rate of work of M1 was also slower. This experiment was repeated nine times, with three different ants for each experiment. The data obtained from these nine experiments, together with those of the first experiment, are summarized in Tables 7 and 8.

Table 7 shows the reaction time of the individual workers. Table 8 shows the number of earth-pellets carried out of the nests by each individual. In the experiments numbered 1, 7, 8, 9, 10, M worked with a rapid co-worker in the afternoon but with a slow co-worker the following morning. In the experiments numbered 2, 3, 4, 5, and 6 the sequence was reversed, i.e., M worked with a slow co-worker in the afternoon but with a rapid co-worker the next morning. The signs $>$ and $<$ in the middle columns of Tables 7 and 8 indicate that the data recorded under "M(S)" are larger or smaller than the data recorded under "M(R)."

The data presented in Table 7 show clearly that the reaction times of all the 10 medium ants (M) were longer when working with slow co-workers (S) and shorter when

working with rapid co-workers (R). The data in Table 8 show that among the 10 medium ants, 7 carried fewer pellets when working with co-workers who carried fewer pellets, but more pellets when working with co-workers who carried more pellets. From all these results, we may conclude that the rapid co-worker has an accelerating effect and the slow co-worker has a retarding effect upon the work of an individual.

TABLE 6

DIFFERENCE BETWEEN THE EFFECT OF A RAPID CO-WORKER AND THAT OF A SLOW CO-WORKER

Time in Minutes	Total Number of Earth-Pellets Carried by Each Ant			
	M1 Working with a Rapid Co-worker		M1 Working with a Slow Co-worker	
	R1	M1	M1	S1
First hour:				
5	21	17	10	5
10	39	29	23	14
15	50	47	36	25
20	70	67	54	36
25	85	80	76	47
30	111	106	99	63
35	133	122	110	75
40	158	144	124	94
45	184	162	143	110
50	209	182	164	127
55	232	200	179	141
60	255	222	198	152
Second hour:				
5	276	242	217	169
10	302	263	237	183
15	324	286	247	197
20	345	307	269	213
25	374	328	286	226
30	395	349	302	240
35	420	371	321	258
40	439	390	340	276
45	464	413	358	288
50	484	435	377	303
55	503	452	392	318
60	521	470	409	331

EFFECT OF THE SIZE OF THE GROUP ON THE NEST-BUILDING ACTIVITY OF AN INDIVIDUAL ANT

The data in Table 3 show that the amount of accomplished work of ants was 3.649 times more when they worked in 2-ant groups than when they worked in isolation. Table 4 shows that it was 3.456 times more when the ants worked in 3-ant groups. The slight difference between 3.649 and 3.456 indicates that the effect of working in association with one co-worker in the 2-ant group was practically the same as that of working in association with 2 co-workers in the 3-ant group.

In order to find out whether there is any difference between the effect of 1 or 2 co-workers and that of more than 2 co-workers, I made the following three experiments: In the first experiment I selected from a colony of *Camponotus* 2 large workers whose rate of work was intermediate between the rapid and the slow co-workers. One of these, A1, was used as experimental ant; and the other, B1, as control ant (see Table 9). Both

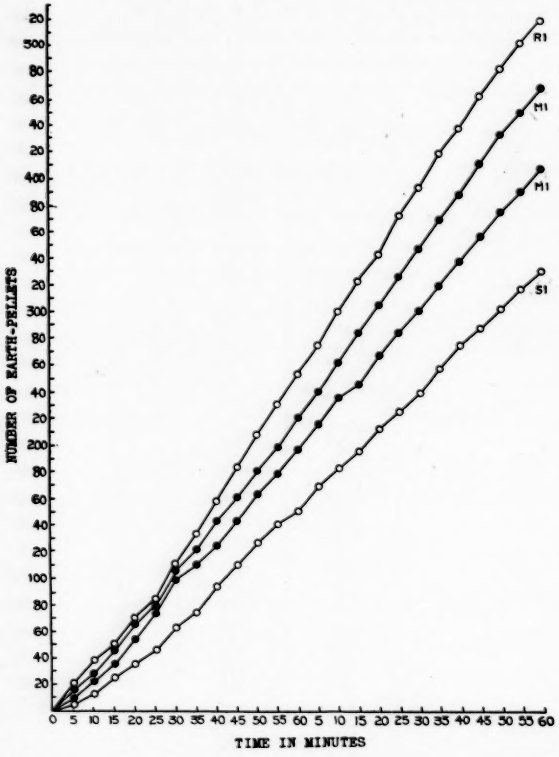

FIG. 2.—Different effect of a rapid co-worker and a slow co-worker on the work of an ant

of them were allowed to work separately in two working dishes (Fig. 3) twice a day for a period of $2\frac{1}{2}$ days and were kept separately in the resting bottles during the remaining hours.

During the period of work, as well as that of rest, B1 was always accompanied by 2 co-workers, one of which was a small rapid ant and the other a small slow ant. Hence the number of ants in the B1 group was always 3. But the number of co-workers accompanying A1 varied from one period of work to another. In the first period of work, A1 was accompanied by 1 rapid co-worker and 1 slow co-worker, the total number of ants in the group being also 3. In the second period of work the number of both rapid

and slow co-workers was 3 each, the total number being increased to 7. By increasing the number of rapid and slow co-workers to 6, 10, and 20 in successive trials, the total number of ants in the group of A1 was increased to 13, 21, and 41.

TABLE 7

DIFFERENT EFFECT OF RAPID AND SLOW CO-WORKERS ON REACTION TIME*

Exper. No.	Reaction Time in Minutes				
	M Working with a Slow Co-worker		Comparison of M(S) with M(R)	M Working with a Rapid Co-worker	
	S	M(S)		M(R)	R
1	3.03	2.77	>	1.93	1.05
2	7.63	7.28	>	0.87	0.88
3	8.05	8.17	>	0.85	0.87
4	58.72	58.48	>	10.47	12.42
5	7.80	7.88	>	4.62	4.67
6	17.57	14.93	>	1.45	1.32
7	61.17	56.30	>	7.28	7.00
8	6.17	1.08	>	0.97	0.92
9	79.75	80.08	>	2.23	77.15
10	5.28	4.62	>	2.18	1.85

* M(S), M when working with S; M(R), M when working with R.

25.52 24.16 3.28 10.81

TABLE 8

DIFFERENT EFFECT OF RAPID AND SLOW CO-WORKERS ON NUMBER OF EARTH-PELLETS*

Exper. No.	Number of Earth-Pellets Carried in 2 Hours				
	M Working with a Slow Co-worker		Comparison of M(S) with M(R)	M Working with a Rapid Co-worker	
	S	M(S)		M(R)	R
1	331	409	<	470	521
2	356	281	<	375	377
3	123	358	>	339	342
4	16	105	<	269	213
5	208	265	>	245	297
6	72	321	<	368	236
7	210	145	<	354	351
8	310	544	>	471	518
9	144	101	<	180	146
10	211	78	<	249	336

198.1 269.7 332.0 333.7

* M(S), M when working with S; M(R), M when working with R.

TABLE 9

RELATION OF GROUP-SIZE TO NEST-BUILDING ACTIVITY*

EXPERIMENT NO. 1

	Date				
	July 30 P.M.	July 31 A.M.	July 31 P.M.	Aug. 1 A.M.	Aug. 1 P.M.
Experimental ant A1:					
Number of co-workers..	1R+1S	3R+3S	6R+6S	10R+10S	20R+20S
Number of pellets.....	392	281	194	199	65
Control ant B1:					
Number of co-workers..	1R+1S	1R+1S	1R+1S	1R+1S	1R+1S
Number of pellets.....	412	353	119	218	276

EXPERIMENT NO. 2

	Date				
	Aug. 3 P.M.	Aug. 4 A.M.	Aug. 4 P.M.	Aug. 5 A.M.	Aug. 5 P.M.
Experimental ant A2:					
Number of co-workers..	1R+1S	10R+10S	15R+15S	20R+20S	25R+25S
Number of pellets.....	341	246	324	238	196
Control ant B2:					
Number of co-workers..	1R+1S	1R+1S	1R+1S	1R+1S	1R+1S
Number of pellets.....	334	233	168	227	207

EXPERIMENT NO. 3

	Date				
	Aug. 6 P.M.	Aug. 7 A.M.	Aug. 7 P.M.	Aug. 8 A.M.	Aug. 8 P.M.
Experimental ant A3:					
Number of co-workers..	1R+1S	3R+3S	6R+6S	10R+10S	20R+20S
Number of pellets.....	53	76	16	92	106
Control ant B3:					
Number of co-workers..	1R+1S	1R+1S	1R+1S	1R+1S	1R+1S
Number of pellets.....	190	206	227	221	278

* R+S, number of rapid co-workers and number of slow co-workers.

An explanation should be added here in regard to the procedure of using equal numbers of rapid and slow co-workers to accompany A1 and B1. The experiments described in the previous section had shown that the effect of a rapid co-worker is different from that of a slow co-worker. Equal numbers of rapid and slow co-workers were used in order to balance the effect of the rapid co-worker by that of the slow co-worker so that the effect of group-size, if there is any effect, could be better exhibited.

The working dishes for A1 and B1 were of equal size and similar form (Fig. 3). They contained well-prepared moist earth favorable for nest-building. Each group of ants was allowed to work in one of these dishes for a period of 2 hours in the morning and another period of similar length in the afternoon. Since A1 and B1 were larger than the co-workers accompanying them, the former could be easily identified among the latter. During the working period, the activity of A1 and B1, in separate dishes, was constantly under observation. With the help of my assistants the numbers of earth-

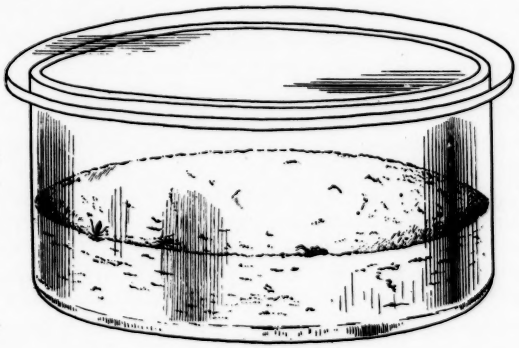

FIG. 3.—Working dish

pellets carried out from the nests by A1 and B1 were separately counted from the time when they started their work to the end of the 2-hour working period. The third experiment was carried out exactly as the first, but the procedure in the second experiment was slightly modified (see Table 9). Here, instead of 1, 3, 6, 10, and 20 rapid and slow co-workers accompanying the experimental ant, the numbers of rapid and slow co-workers were 1, 10, 15, 20, and 25 in the successive trials.

The data obtained in the three experiments mentioned above are summarized in Table 9. In this table the number of earth-pellets recorded for each ant varied from one period of work to another. But, as a rule, the records of the experimental animal and those of the control almost always varied in the same direction, i.e., increased or decreased at the same time. This result indicates the absence of any distinct effect of group-size on the rate of work of the experimental ants. The records of Experiment 1 seem to show that the group-size of 41 ants caused a great decrease in the number of pellets carried by A1, but similar group-size in Experiments 2 and 3 did not produce the same effect on A2 and A3. From the data in Table 9, as well as those in Tables 3 and 4, we may conclude that there is no difference between the effect of a group of 2 ants and that of a group of many more ants on the rate of work of the individual ant within the group.

REVIEW OF LITERATURE AND DISCUSSION

The modification of the activity of individuals by social environment has long been studied in the field of human social psychology. Triplett, Mayer, Moede, Allport, and others (Allport, 1924; Murphy and Murphy, 1931) found that, in both physical and mental work, children or adults accomplish more work, and with greater uniformity or less deviation, when working in association with other co-workers than when working in isolation. They found that the work of an individual within a group is accelerated by a more rapid co-worker but retarded by a slower one, and that the accelerating effect of association is greatest for the slowest and least for the most rapid members of the group because the average speed of activity of the group is more rapid than the speed of the slow members of the group but less rapid than that of the rapid members of the group. According to Allport (1924), two social factors may be recognized in the social influence on activity of individuals. "The first is social facilitation, which consists of an increase of response merely from the sight or sound of others making the same movements. The second is rivalry, an emotional reinforcement of movement accompanied by the consciousness of a desire to win."

While the social influence on the activity of individuals has been much studied in man, it is very little explored in other animals. Bayer (1929) found that hens eat more when in groups than when isolated. In 4-hen groups the social increment ranged from 33 per cent to 200 per cent, and averaged 96 per cent.

Harlow (1932) investigated the social facilitation of feeding in the albino rat and found that the amount of food eaten by 20 rats was 70 per cent more when these were fed in groups of 5 than when fed individually. In many cases, however, the difference between the experimental and the control animals were just statistically significant. Harlow also observed that social facilitation is not a function of the size of the group, the results of the experiments on groups of 5 rats, groups of 2 rats, and groups of 3 rats being similar. Here, too, the numerical data which the author reported seem too few to substantiate his conclusion.

In fishes, Welty (1934) found that goldfishes become conditioned to run a simple maze more rapidly when gouped than when isolated; the larger the group the quicker the conditioning. He also found that a trained goldfish in a maze with an untrained fish will speed up the conditioning of the latter. In some experiments the fish which gave the most rapid reaction was found among the isolated fishes and not in the groups. Likewise the slowest fish to respond was found among the isolated fishes. This shows a greater variation in reaction time among the isolated fishes. Concerning social facilitation in feeding, Welty found that fishes eat more food per fish in groups than when isolated. He also observed that under certain experimental conditions mud minnows in groups exhibit an antagonistic behavior that inhibits learning.

Although the social life of ants has long been studied in detail by numerous entomologists, no one has yet investigated the problem of social modification of individual activities in ants as Triplett, Mayer, Allport, Bayer, Harlow, Welty, and others studied this problem in man, hens, rats, and fishes. The experimental results reported in the present paper agree essentially with the principles established by earlier workers for man and other animals. They agree with the work on men, hens, rats, and fishes in that individuals react more rapidly and accomplish more when in groups than when isolated. However, social increment in ants is much greater than in man or other animals. When the ants worked in 2-ant or 3-ant groups, the reaction time was, on the average, 28+

minutes or 33+ minutes; when they worked in isolation, the reaction time was 192+ minutes or 160+ minutes. The isolated ants excavated, on the average, 0.207 gm. of earth per ant in 6 hours. When grouped, the amount of excavated earth was greatly increased; it was 3.649 times more when the ants worked in 2-ant groups and 3.456 times more when they worked in 3-ant groups than when they worked in isolation. The social increment in man and other animals is never so great as that of ants, in some cases the difference between the data of the grouped individuals and those of the isolated individuals being only just statistically significant. The very large social increment in ants is probably due to the fact that ants are much more highly socialized than men, hens, rats, and goldfishes; hence they are much more affected by being isolated or grouped.

In ants, I observed the fact that the grouped individuals worked with greater uniformity than the isolated individuals, that the accelerating effect of association was greater for the slower workers than for the more rapid workers, and that the more rapid co-worker has an accelerating effect, and the less rapid co-worker has a retarding effect, upon the work of an individual within a group. Mayer and others (Allport, 1924; Murphy and Murphy, 1931) observed similar facts in their studies of human social behavior; and Welty (1934), in his experiments on goldfish.

In regard to the effect of the size of the group on the activity of an individual, the experimental data which I obtained show that there is no difference between the effect of a group of 2 ants and that of a group of many more ants on the rate of work of an individual ant within the group. This problem has not yet been extensively investigated in man and other animals. Incidentally, contradictory results have been obtained by Harlow (1932) and Welty (1934) in their studies on rats and goldfishes. Harlow obtained results, which agree with my observations, showing that the social facilitation of feeding in the albino rat was not a function of the size of the group, but Welty found that the larger the group the quicker the goldfishes become conditioned to run a simple maze.

SUMMARY

The author reports in this paper the results of studies on the social modification of the activity of individuals in a variety of common Asiatic ants, *Camponotus japonicus* var. *aterrimus* Emery. From the experimental data the following conclusions were derived:

1. All the workers of *Camponotus* are capable of nest-building in isolation, but building work varies with different individuals.
2. The reaction time is shorter when the ants work in association than when they work in isolation.
3. The amount of work accomplished is more when the ants work in association than when they work in isolation.
4. The associated ants work with greater uniformity then the isolated ants.
5. The accelerating effect of association is greater for the slow workers than for the rapid workers.
6. The rapid co-worker has an accelerating effect, and the slow co-worker has a retarding effect, upon the work of an individual.
7. There is no difference between the effect of a group of 2 ants and that of a group of many more ants on the rate of work of the individual ant within the group.
8. The results of the present study in ants, as reported above, agree in principle with those of similar studies in men, hens, rats, and goldfishes.

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THE LEADERS AND FOLLOWERS AMONG THE ANTS IN NEST-BUILDING

(Five figures)

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DURING my study of the social modification of the activity of individual ants (Chen, 1937) I noticed the fact that the ability of nest-building varies with different ants. Some ants are more active than others and in favorable conditions start their work of nest-building very early. Others are less active and usually follow the more active ants in selecting a place for nest-building and in starting work. In a group of a few or numerous ants the one which first starts the building work may be regarded as the leader of the group because it is through the initiative of this ant that the site of their nest is determined and the co-operative work of the whole group is initiated. Other ants within this group may be regarded as followers of this leader.

It is the purpose of the present investigation to study the relationship between the leaders and the followers in ants, to examine the morphological and physiological differences between the leaders and the followers, to study the nest-building activity of isolated leaders and followers, to form artificial leader-groups and follower-groups, and to study the behavior of these groups as a whole.

The material which I used for the present study is the same as that used for my previous study, i.e., *Camponotus japonicus* var. *aterrimus* Emery. All the experiments to be reported were made during the summer and autumn of 1936 in the laboratory of the Goldfish Garden of the National Tsing Hua University. Details concerning the material, laboratory, and methods were reported in my previous paper (Chen, 1937).

THE LEADER-FOLLOWER ORDER

The leader-follower order of ants selected at random.—From a healthy colony of *Camponotus* I selected at random six small workers, six medium-sized workers, and six large workers. The small workers varied in body length from 7.2 mm. to 9.7 mm. and were named "S1," "S2," . . . . , "S6." The medium-sized workers varied in body length from 10.2 mm. to 11.6 mm. and were named "M1," "M2," . . . . , "M6." The large workers varied in body length from 15.4 mm. to 16.4 mm. and were named "L1," "L2," . . . . , "L6." These eighteen ants were kept in separate vials, each of which contained a small piece of moist sponge and was plugged with a small piece of cheesecloth.

I took these ants two by two and introduced them into working dishes (Chen, 1937, Fig. 3), i.e., shallow glass dishes with glass covers and containing moist earth to the depth of about 1 inch. The ants in the working dishes at first explored the new environment for a little while and then selected a favorable place and started their nest-building work. In any one of the two-ant groups there was one ant which initiated the nest-building work and was thus the leader of the group while the other ant was the follower.

I made numerous different combinations and recombinations of the eighteen ants, always taking two ants at a time, and determined, by the method described above, which

one of the two ants was the leader and which the follower. The results which I obtained from these determinations are summarized and graphically shown in Figure 1.

Figure 1 shows the names of sixteen ants arranged in a linear order, with M_4 at the top and L_2 at the bottom. The position of M_4 indicates that it is the leader in its relation to all the other fifteen ants below it. The low position of L_2 means that it is a follower of all the other ants above it. The ants arranged in the middle of the scale are leaders of all the ants below them but followers of all those above them. Thus, M_3 is a follower in its relation to M_2, M6, L_1, L_3, etc., but a leader of L6, S_3, S_1, S_5, etc. The long vertical line at the left represents the scale of leader-follower order: the higher the position on the scale, the higher is the leadership; the lower the position on the scale, the lower is the followership. The horizontal lines indicate the relative positions of the sixteen ants on the scale of leader-follower order. The numerous short and long vertical lines at the right show the results of numerous experiments by which the leader-follower relationships were determined. Thus, from the right side to the left, the first vertical line connects the horizontal line of L_3 with that of S_1. This means that an experiment had been made by combining L_3 and S_1 together to form a two-ant group and allowing this group to build a nest in a working dish; the result of this experiment was that L_3 was the leader, S_1 being the follower. The second vertical line means that M_5 had been experimentally determined to be the leader in its relation to L_5, and the third vertical line means that M_4 had been similarly determined to be the leader to L_4. The forty-one vertical lines indicate the results of forty-one different determinations of the leader-follower relationship between the ants whose horizontal lines are connected by these vertical lines.

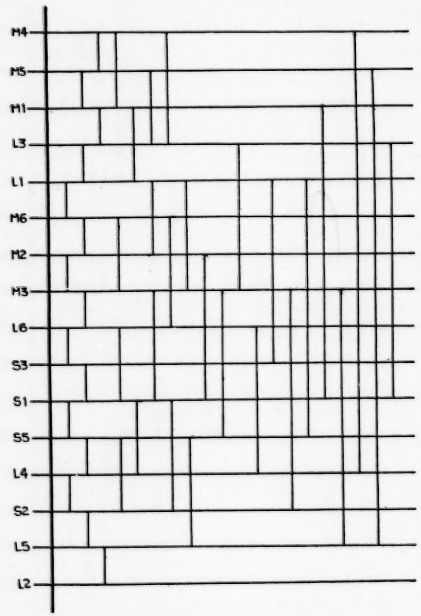

FIG. 1.—Linear order of leadership

The absence of contradictions between the results of the forty-one experiments mentioned above is good evidence to show the real existence of a linear order in the leader-follower relationship and that the positions of the sixteen ants in Figure 1 were correctly determined.

There are two ants whose names are not arranged on the scale; these are S6 and S_4. S6 was a very inactive ant and did not start the nest-building work within 1 hour after the introduction into the working dish. Since the maximum experimental period of all the experiments planned for the determination of the leader-follower order was limited to 1 hour, the position of S6 could not be determined, especially in relation to L_2, which was also a very inactive ant. S_4 was accidentally injured during the experiments and hence was discarded.

The experiments reported above were made on July 1. A similar series of experiments

was made earlier (i.e., on May 31) on twelve ants of different size. The results of thirty-one tests made on these ants showed that all of them might be similarly arranged according to their leader-follower relationship in the form of a linear scale.

In order to find out whether the linear order of leader-follower relationship remained constant or not, I made the following two series of experiments; all of them yielded similar results, showing that the order is not constant in successive days.

The first series of experiments was made on six ants of various size, to each of which a permanent number was given. It was started on August 3 and ended on August 10. Based upon numerous tests, the order of the six ants was determined to be 1,6,5,4,3, and 2 on August 3, 1 being the highest leader and 2 the lowest follower. This order was determined again by numerous tests on the next day; the order had been changed to 1, 6, 5, 4, 2, and 3. By making many tests in successive days, I found that the order was 1, 6, 2, 3, 4, and 5 on August 5; 1, 6, 4, 2, 3, and 5 on August 6; 1, 4, 6, 2, 5, and 3 on August 7; 1, 4, 2, 6, 5, and 3 on August 8; and 1, 5, 6, 3, 2, and 4 on August 10.

The second series of experiments was made on seven ants, each of which was permanently numbered at the beginning of the experiments. This series of experiments was started on August 11 and ended on August 14. The order of these ants in successive days was 5, 6, 2, 1, 7, 4, and 3 on August 11; 6, 1, 2, 3, 7, 5, and 4 on August 12; 1, 2, 5, 6, 3, 4, and 7 on August 13; and 6, 2, 3, 1, 7, 5, and 4 on August 14.

The apparatus for selecting the most active leaders and the most inactive followers of a group.—In spite of the fact that the leader-follower order of any two ants selected at random was found to be inconstant, it was suspected that the order between the most active leader and the most inactive follower selected from a group of ants might be constant in successive daily trials. In order to carry out experiments along this line, it was necessary at the beginning to select the most active leader and the most inactive follower from a group of one hundred or more ants. The selection of such leaders and followers was made by means of an apparatus as illustrated by Figure 2 and described below:

This apparatus is a square wooden box (Fig. 2, *W.B.*) whose inner dimensions are 2 feet on each side and 1 foot in depth. Inside this box there are four glass vessels (*G.V.*), about $4\frac{1}{2}$ inches in height and 3 inches in diameter, each of which is arranged at one corner of the box. These vessels contain well-prepared moist earth to the full height of the vessel. They are buried in a thick layer of dry and compact earth (*D.E.*), which covers the whole bottom of the box as deep as the height of the vessels. The moist earth within the vessels and the dry earth outside are made as nearly as possible on a level with the rims of the vessels. The box is covered at the top by two movable pieces of glass (*G.C.*) which fit tightly between each other as well as between the glass covers and the wooden rim of the box.

Before starting the selection of leaders and followers, a group of ants amounting to one hundred or more is transferred into a round glass dish about 5 inches in diameter and 4 inches in height. This dish has a flat bottom and a round flat glass cover. The inner surface of the dish is perfectly dry, and a very thin layer of fine dry earth has been sprinkled over it so that when the dish with its glass cover on it is turned upside down the majority of the ants in it cannot stick to the inner surface of the bottom and the side of the dish but fall down on the glass cover. In this inverted position the glass dish (*G.D.*) becomes a cover which lies on a piece of round glass (*C.P.*) and keeps the ants from running away from the latter.

The glass dish in its inverted position with a group of ants in it is now carefully put

at the center of the box on the surface of the dry earth. After the ants are quieted from the agitation caused by the inversion and the transference of the dish, the latter is carefully removed from the round piece of glass which still lies in the middle of the box on the surface of the earth. This round piece of glass is now known as "central plate" (*C.P.*). By shaking the dish gently, all the ants which are attached to the inner surface of the dish are made to fall on the central plate. After the glass dish is taken out of the box, the glass covers (*G.C.*) of the box are quickly shifted to the middle, so that the box is tightly covered, ventilation being maintained by a small screened window at the side of the box. The ants are now released from their inclosure and allowed to run and to explore freely within the box.

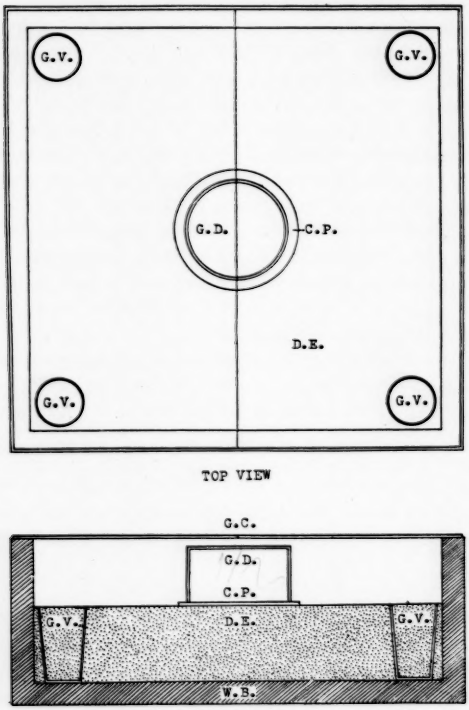

FIG. 2.—Wooden working arena. *C.P.*, central plate; *D.E.*, dry earth; *G.C.*, glass cover; *G.D.*, glass dish; *G.V.*, glass vessel; *W.B.*, wooden box.

The following is a brief description of the behavior of a group of ants within the apparatus as frequently observed: As soon as the glass dish or cover is removed, a few of the very active members of the group begin their journey of exploration. They leave the central plate and the group to which they belong and wander separately and independently in all parts of the unknown world in the box. As time goes on, the number of ants remaining on the central plate gradually decreases, while the number of exploring ants gradually increases. A few minutes after the removal of the glass dish, when the majority of the ants are still on the central plate, one or two of the most active ones begin their work of nest-building in one of the corners of the box on the moist earth contained in a glass vessel. There are four similar vessels situated in four corners of the box; hence there are four places in the arena equally favorable for nest-building. While the leaders in one of these four places attract a great many followers and are busy in their building work, one or a few ants may work independently in another corner of the box. About 1 hour after the removal of the glass cover, there may be two or three co-operating groups working in two or three different corners of the box. At this time there are still some ants remaining sluggishly on the central plate and some ants wandering aimlessly in the arena, often passing the places where others are working energetically without taking part in the nest-building work.

The procedure for selecting the most active leaders and the most inactive followers from a group was as follows: From a colony of *Camponotus* I selected at random one hundred healthy individuals to form a group. I transferred this group into a glass dish, inverted the dish, and placed the dish in the center of the wooden arena. After the removal of the glass dish, the most active ants left the central plate, went to a corner of the wooden arena, and began to build a nest there. When the first ant began its building work, I caught it by means of a small vial, leaving the other ants in the arena as little disturbed as possible. Soon the second ant began its building work and I caught it also. In this way I caught the successive initiators of building work until the tenth one was caught. These captured ants were the most active members or leaders of the group. While the active ants were building or wandering in the arena, the sluggish ones remained in the center of the arena on the central plate. I caught the last ten ants remaining on the central plate. These were the most sluggish members or followers of the group.

The leader-follower order of the most active and the most inactive members of a group.—From a colony of *Camponotus* I selected at random four hundred sound and healthy workers and divided them into four groups—A, B, C, and D—each of which consisted of one hundred workers. I introduced Group A into a wooden arena and selected, according to the procedure described above, ten most active leaders and ten most inactive followers. By combining one leader with one follower, I made ten leader-follower groups out of the leaders and followers selected from the Group A. These ten groups were numbered $A_1, A_2, \ldots, A_{10}$. Similarly, I selected ten most active leaders and ten most inactive followers from Group B, C, D, respectively, and formed leader-follower groups from each of these 100-ant groups. These leader-follower groups were numbered $B_1, B_2, \ldots, B_{10}, C_1, C_2, \ldots, C_{10}$, and $D_1, D_2, \ldots, D_{10}$ (Table 1).

In combining one leader with one follower to form a leader-follower group, care was taken to see that there was a distinct difference in the body size of these two ants and to note down this relative difference so that it would be possible to identify which one of them was the original leader and which the original follower in the later daily trials.

Each of the forty leader-follower groups was kept in a separate glass bottle, which was covered by a piece of cheesecloth and which contained a piece of wet sponge but no moist earth. From these bottles they were taken out for daily determinations ofthe leader-follower order. These determinations were made in shallow glass dishes (Chen, 1937, Fig. 3) containing well-prepared most earth favorable for nest-building. When the leader-follower order was the same as the original one, i.e., the leader was the original leader and the follower the original follower, the result of the determination was recorded by the plus sign (+) (Table 1). In case the order, as determined by a daily trial, was a reversal of the original one, this result was recorded by the minus sign (−).

Table 1 shows the results of 200 successive daily trials of the forty leader-follower groups. Among the 200 signs in this table, there are 161 plus signs and 39 minus signs. According to the number of "+" or "−" signs belonging to each group, these forty groups are distributed into the classes shown in Table 2. In the first class there are eighteen groups, or about one-half of all the groups, each of which has 5 "+" signs. These results show that the distinction of leaders and followers in these groups is based upon a permanent large difference in the ability or activity of the ants in nest-building. In the second class there are twelve groups, or about one-quarter of all the groups, each of which has 4 "+" and 1 "−." These results show that most probably the dis-

tinction of leaders and followers in these groups is also based upon a permanent difference in the activity of ants. In the third, fourth, and fifth classes there are ten groups, or one-quarter of all the groups, each of which has 3, 2, or 1 "+" and 2, 3, or 4"−." These results show that probably there is no permanent distinction between the leaders and the followers in these ten groups, the leader-follower order in the successive trials being due to a temporary small difference in the physiological condition of the individuals.

TABLE 1

LEADER-FOLLOWER ORDER IN SUCCESSIVE DAILY TRIALS

Group No.	Trial No.					Group No.	Trial No.				
	1	2	3	4	5		1	2	3	4	5
A1	+	+	+	+	+	C1	+	+	−	−	+
A2	+	+	+	+	+	C2	+	+	+	+	+
A3	+	+	+	+	+	C3	+	+	+	−	−
A4	+	+	+	+	+	C4	+	−	−	+	−
A5	−	−	+	−	+	C5	+	+	+	+	+
A6	−	+	+	+	+	C6	+	+	+	+	+
A7	+	+	+	+	−	C7	+	+	+	+	+
A8	+	+	+	+	+	C8	+	+	+	−	+
A9	+	+	−	+	+	C9	+	+	+	+	+
A10	+	+	+	+	−	C10	+	+	−	+	+
B1	+	+	−	−	−	D1	+	+	−	+	+
B2	+	+	+	+	+	D2	+	+	+	+	+
B3	+	+	−	+	+	D3	+	+	+	+	−
B4	+	+	+	+	+	D4	−	−	−	+	+
B5	+	−	+	+	+	D5	+	+	+	+	−
B6	+	+	+	+	−	D6	+	+	+	+	+
B7	+	+	−	−	−	D7	+	+	+	+	+
B8	+	+	+	+	+	D8	−	+	+	+	−
B9	+	+	−	−	+	D9	−	−	−	+	−
B10	+	+	+	+	+	D10	+	+	+	+	+

TABLE 2

Class No.	No. of Groups
1. With 5 "+" and 0 "−"	18
2. With 4 "+" and 1 "−"	12
3. With 3 "+" and 2 "−"	4
4. With 2 "+" and 3 "−"	5
5. With 1 "+" and 4 "−"	1
Total	40

The results of daily trials of forty groups in the five successive days may be also summarized according to the number of "+" and "−" signs in each day, as shown in Table 3. The data presented show that there is a distinct decrease of "+" and increase of "−" in the third, fourth, and fifth trials.

In another series of similar experiments, I also made forty leader-follower groups from the ants selected from four 100-ant groups. In this case, the daily trials were continued

for 7 days; hence, there are 7 "+" signs for each leader-follower group and 280 signs for all the forty groups. Among these 280 signs, there are 207 "+" and 73 "−." According to the number of "+" and "−" signs, these forty groups are distributed in the classes given in Table 4. By combining the first three classes together, we get twenty-nine groups, or about three-quarters of all the groups, each of which has 7, 6, or 5 "+" and 0, 1, or 2 "−." By combining the Classes 4–8 together, we get eleven groups, or about one-quarter of all the forty groups, each of which has 4, 3, 2, 1, or 0 "+" and 3, 4, 5, 6, or 7 "−." Hence, the results of this series of experiments agree with those obtained in the earlier experiments.

TABLE 3

Trial No.		
1	35 "+" and	5 "−"
2	35 "+" and	5 "−"
3	29 "+" and	11 "−"
4	33 "+" and	7 "−"
5	29 "+" and	11 "−"
Total	161 "+" and	39 "−"

TABLE 4

Class No.	No. of Groups
1. With 7 "+" and 0 "−"	15
2. With 6 "+" and 1 "−"	8
3. With 5 "+" and 2 "−"	6
4. With 4 "+" and 3 "−"	2
5. With 3 "+" and 4 "−"	2
6. With 2 "+" and 5 "−"	4
7. With 1 "+" and 6 "−"	2
8. With 0 "+" and 7 "−"	1
Total	40

The numbers of "+" and "−" signs in the trials of all the forty groups in the successive days are given in Table 5. Contrary to the results of earlier experiments, the data presented above show that the number of "+" and "−" signs changed very little in the successive days.

Conclusions.—Basing upon the results of all the experiments reported above, we may safely draw the following conclusions: Individual ants vary in their activity in nest-building. This variation is a continuous gradient; hence there are all sorts of gradations in the activity of individual ants, ranging from the most sluggish to the most active ones. When two ants are working together in nest-building, the comparatively more active ant becomes the leader, the less active one being the follower. In case the difference in the relative activity of these two ants is very small, such as in most of the cases when we select any two ants at random, this difference is very easily affected by any temporary change in the physiological condition of the individuals; hence the leader-follower order of these two ants is constant for only a short period of few hours and is continuously changing in the successive daily trials. When the difference in the relative activity of two ants is larger, it is less liable to be reversed by temporary physiological

changes, and the leader-follower order of these ants is more or less constant. When the difference in the relative activity of two ants is very large, as found in most of the leader-follower groups formed by ants selected from a large group of ants, this difference is very difficult to eliminate or reverse by a temporary change in the physiological condition of the individuals; hence the leader-follower order of the ants is constant in most or all of the successive daily trials.

The difference in the relative activity of any two ants selected at random is more likely to be small and their leader-follower order is more likely to be inconstant. Consequently, in selecting leaders and followers for study, it is necessary to select the most active leaders and the most inactive followers from a large group of ants because the difference in the activity of the leaders and the followers thus selected is more likely to be large and their leader-follower order more likely to be constant.

TABLE 5

Trial No.	
1	30 "+" and 10 "−"
2	29 "+" and 11 "−"
3	30 "+" and 10 "−"
4	34 "+" and 6 "−"
5	29 "+" and 11 "−"
6	26 "+" and 14 "−"
7	29 "+" and 11 "−"
Total	207 "+" and 73 "−"

THE MORPHOLOGICAL DIFFERENCES BETWEEN THE LEADERS AND THE FOLLOWERS

The workers of *Camponotus* vary greatly in body size. In order to find out whether there is any correlation between the body size and the activity or leadership of these workers, the following experiments were carried out:

From a colony of healthy *Camponotus* I selected forty small workers, forty medium-sized workers, and forty large workers. The small workers measured less than 10 mm. in body length, medium-sized workers measured from 11 mm. to 13 mm., and large workers measured above 14 mm. These three classes were clearly separated, and all the one hundred and twenty selected ants could be easily classified into any one of these classes because the ants of intermediate size between small and medium or medium and large classes were not selected.

All the three classes of ants were mixed together to form a 120-ant group. This group was introduced into a wooden arena in which the most active leaders and the most inactive followers were selected according to the procedure described in the previous section. The ten leaders and ten followers thus selected were classified according to their body length into three classes. As a result, I found that among the ten leaders, there were five small workers, four medium-sized workers, and one large worker; and among the ten followers, there were two small workers, three medium-sized workers, and five large workers (Table 6). Thus, it was found that among the leaders one-half were small workers and among the followers one-half were large workers.

The above described experiment was repeated five times, always selecting leaders and followers from a new 120-ant group in each experiment. The results of all these experiments, together with the first one, are summarized in Table 6. In this table, the data

show clearly that the result of the first experiment was confirmed; more than one-half of all the leaders are small workers and more than one-half of all the followers are large workers. Hence, the activity of these ants is correlated to some extent negatively with their body size; the small workers are in general more active than the medium-sized workers, the latter in general more active than the large workers.

THE PHYSIOLOGICAL DIFFERENCES BETWEEN THE LEADERS AND THE FOLLOWERS

Difference in the susceptibility to chloroform and ether.—The difference between the leader and the follower in the ants is a difference in their activity. This is equivalent to saying that the leaders and followers differ in their metabolic rate, the former being higher in metabolic rate than the latter.

TABLE 6

DIFFERENCE IN BODY SIZE BETWEEN LEADERS AND FOLLOWERS IN ANTS

Trial No.	Leaders				Followers			
	Small	Medium	Large	Total	Small	Medium	Large	Total
1	5	4	1	10	2	3	5	10
2	5	2	3	10	1	4	5	10
3	8	2	0	10	3	5	7	15
4	6	2	2	10	2	5	5	12
5	6	1	3	10	2	2	7	11
6	5	3	2	10	2	3	8	13
Totals	35	14	11	60	12	22	37	71

The graded difference in the metabolic rate between the leaders and the followers is a fact which is foreshadowed by the metabolic gradient theory of Child (1924). In proving his theory that the dominant region of the body of an organism is higher in metabolic rate than other parts of the body, Child relies in part upon the evidences derived from his susceptibility experiments. According to him, the higher the metabolic rate of a body part, the more susceptible it is to the injurious effects of many chemical reagents, such as potassium cyanide, chloroform, ether, etc. In order to find out whether there is also a difference in the susceptibility to chemical reagents between the leaders and the followers in ants, as suggested by Child's theory, I planned and carried out the following experiments:

Figure 3 shows the apparatus which I used in testing the relative susceptibility of ants to volatile chemicals. It is a large shallow glass dish measuring 22 cm. in diameter and 11 cm. in height and containing a metallic stand on which are two small inverted glass dishes. These inverted dishes are 10 cm. in diameter and 5 cm. in height. They are covered by pieces of cheesecloth, which are fastened to the dishes by means of rubber bands. There are two large round openings on the stand. Each one of these openings measures 9 cm. in diameter. The inverted dishes are so arranged on the stand that the cheesecloth covers of the two dishes are set against the two openings of the stand. The large glass dish is covered by a round flat glass cover. On the rim of the large glass dish

a thick layer of vaseline is smeared, so that when the cover is put upon it the dish is sealed.

The procedure is as follows: Take out the metallic stand and the small glass dishes from the large dish. Pour a little chloroform or ether into the large dish so that the bottom of the latter is evenly covered by a thin layer of the liquid. Put the stand into the dish and the glass cover upon it. Make sure that the dish is tightly sealed by vaseline so that the volatile chemicals cannot escape from it. Set the apparatus aside for a short time so that the air contained in the dish will be saturated with the volatile chemicals. Meantime select ten most active leaders and ten most inactive followers from a 100-ant group in a wooden arena. This is repeated three times, so that forty leaders and forty followers were obtained from four 100-ant groups. Put the forty leaders into one of the

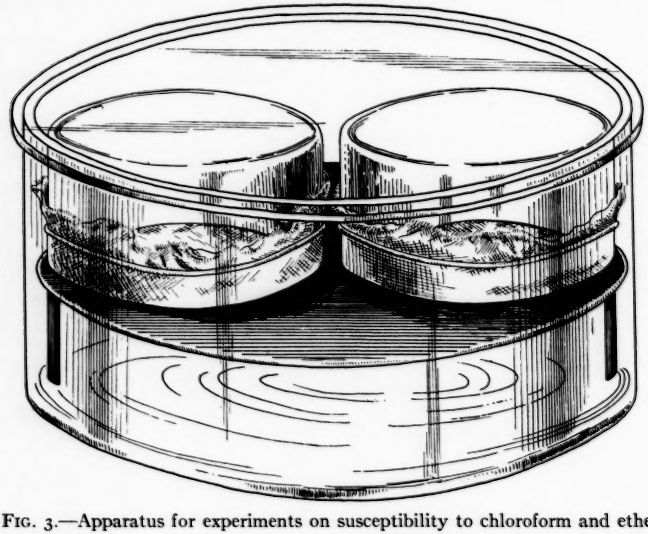

FIG. 3.—Apparatus for experiments on susceptibility to chloroform and ether

small dishes taken out of the apparatus, cover the dish with a piece of cheesecloth, and mark the dish with a wax pencil. Put the forty followers into another small dish; cover and mark it. Invert these two dishes. The dishes are now ready to be introduced into the apparatus. Quickly open the glass cover of the apparatus; introduce the two inverted dishes into the apparatus at the same time; arrange the inverted dishes so that their cheesecloth covers are set against the openings of the metallic stand; close the apparatus by setting the glass cover on it. The above-mentioned steps should be performed as rapidly as possible, preferably by two experimenters working together. The volatile chemicals in the apparatus penetrate through the cheesecloth covers into the inverted dishes. Soon the ants in the inverted dishes show signs of discomfort and suffering. A little later some ants are lying on the cheesecloth. The number of these increases as time goes on. The experimenter should now be very watchful for a difference in the ants inclosed in the two inverted dishes: the leaders are more susceptible to the volatile chemicals than the followers, and lie on the cheesecloth earlier. The difference between the leaders and the followers is observable only during a short time. A few seconds later

all the ants in both dishes lie on the cheesecloth, the difference between the leaders and the followers being now unobservable. Before this stage is reached, and as soon as we observe a maximum difference between the leaders and the followers in regard to the number of ants lying on the cheesecloth, the two inverted dishes should be simultaneously taken out of the apparatus. These dishes should now be opened and the ants in them be classified into three classes: walking, standing but unable to walk, and lying.

TABLE 7

DIFFERENCE IN SUSCEPTIBILITY TO CHLOROFORM BETWEEN LEADERS AND FOLLOWERS

Trial No.	Time Exposed to Chloroform (Seconds)	Leader				Follower			
		Walking	Standing	Lying	Total	Walking	Standing	Lying	Total
1	95	2	6	32	40	22	16	2	40
2	87	4	12	24	40	12	22	6	40
3	45	5	7	28	40	19	15	6	40
4	65	7	9	24	40	19	15	6	40
5	56	7	12	21	40	21	13	6	40
Total	.....	25	46	129	200	93	81	26	200
Percentage	.....	12.5	23	64.5	.....	46.5	40.5	13	.....

TABLE 8

DIFFERENCE IN SUSCEPTIBILITY TO ETHER BETWEEN LEADERS AND FOLLOWERS

Trial No.	Time Exposed to Ether (Seconds)	Leader				Follower			
		Walking	Standing	Lying	Total	Walking	Standing	Lying	Total
1	166	6	8	26	40	26	6	8	40
2	122	16	17	7	40	34	1	5	40
3	81	11	13	16	40	34	5	1	40
4	132	6	7	27	40	27	10	3	40
5	140	5	10	25	40	24	14	2	40
Total	.....	44	55	101	200	145	36	19	200
Percentage	.....	22	27.5	50.5	.....	72.5	18	9.5	.....

The number of ants in each class should be counted and recorded. These steps should also be performed as rapidly as possible, preferably by two experimenters working together, because the ants are gradually recovering from the anesthetization. The time elapsed from the introduction of the inverted dishes into the apparatus to the removal of them from the apparatus is measured by a stop watch.

By means of the apparatus and procedure described above, I carried out five trials on the susceptibility of leaders and followers to chloroform and a similar number of trials on the susceptibility to ether. The data which I obtained from these experiments are presented in Tables 7 and 8. The data show clearly that, in agreement with Child's

theory of metabolic gradient, the leaders are more susceptible to chloroform and ether than the followers. While 12.5 per cent of the leaders in the experiment on the susceptibility to chloroform are walking and 64.5 per cent are lying, there are 46.5 per cent of the followers walking and 13 per cent lying (Table 7). While 22 per cent of the leaders in the experiment on the susceptibility to ether are walking and 50.5 per cent lying, there are 72.5 per cent of the followers walking and 9.5 per cent lying (Table 8). The time when the ants were exposed to chloroform for maximal difference between the leaders and the followers was, on the average, shorter than the time exposed to ether.

Difference in the ability to resist drying and starvation.—On several occasions I observed incidentally that the death-rate of the leaders of the ants is higher than that of the

TABLE 9

DIFFERENCE IN SUSCEPTIBILITY TO DRYING BETWEEN LEADERS AND FOLLOWERS

Date	Percentage Died		Percentage Living		Difference between Leaders and Followers
	Leaders	Followers	Leaders	Followers	
Sept. 3	0	0	100	100	0
4	25	2	75	98	23
5	58	5	42	95	53
6	74	10	26	90	64
7	83	19	17	81	64
8	85	25	15	75	60
9	89	25	11	75	64
10	94	27	6	73	67
11	97	36	3	64	61
12	97	45	3	55	52
13	98	52	2	48	46
14	99	69	1	31	30
15	99	79	1	21	20
16	99	87	1	13	12
17	100	92	0	8	8
18	100	98	0	2	2
19	100	100	0	0	0

followers. This difference in the death-rate was confirmed by three preliminary experiments, the result of which showed that the leaders are much more susceptible to drying and starvation than the followers. Finally, I planned and carried out the following two experiments for additional data concerning this difference in the ability to resist drying and starvation between the leaders and the followers:

From two colonies of *Camponotus* I selected five groups of ants, each consisting of one hundred and fifty workers. I transferred these groups, one by one, into the wooden arena. Then I selected twenty most active leaders and twenty most inactive followers from each of these groups. Thus I obtained one hundred leaders and one hundred followers. I put these leaders and followers into two hundred vials, which were 8 cm. in length and 2 cm. in diameter, each containing one leader or one follower. These vials contained neither food nor water and were loosely plugged with a double layer of cheesecloth. They were placed on a laboratory desk in a place free from any disturbing effect of direct sunlight, sudden temperature-change, volatile chemicals, etc. I examined

these vials three times a day to find out whether there were any dead ants; and if dead ants were found, they were recorded.

The records concerning the death of the ants due to drying, as well as the number or percentage of ants still living, were presented in Table 9. The number of ants still living is also graphically shown in Figure 4. According to the data in Table 9 and Figure 4, it is clear that death due to drying is much quicker in the leaders than in the followers. The difference between the number of the surviving leaders and that of the followers is especially great during the early and the middle parts of the experimental period.

The experiment on the difference in the ability to resist starvation between the leaders and the followers was carried out in the same way as the experiment on the resistance to drying described above, but with the following modification: I put a piece of sponge, washed and soaked in distilled water, into each of the one hundred vials containing the

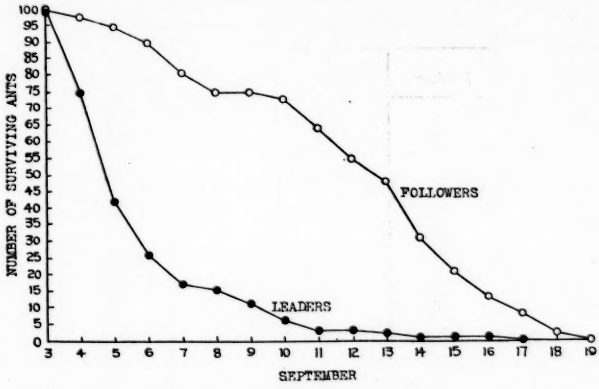

FIG. 4.—Susceptibility to drying

leaders and the same number of vials containing followers. These pieces of wet sponges were used for keeping the interior of the vials in a moist condition favorable for the life of ants, and distilled water was frequently added to keep the sponge constantly wet.

The records which I obtained in the experiment on resistance to starvation are presented in Table 10, and the numbers of the surviving leaders and followers are graphically shown in Figure 5. The graphs and data show clearly that, as in the case of the experiment on the resistance to drying, death due to starvation was also much quicker in the leaders than in the followers. The maximum difference between the number of the surviving leaders and that of the surviving followers was seventy-two, at the time when the number of the surviving leaders was twelve and that of the surviving followers was eighty-four. This is not significantly different from the data in Table 9, which shows that the maximum difference was sixty-seven, at the time when the number of the surviving leaders was six and that of the followers was seventy-three.

A comparison of the data in Table 9 with those in Table 10 shows that death due to starvation is a much slower process than death due to drying. While all the ants died, on account of drying, within a period of 16 days, seventy-nine of the one hundred followers were still living 40 days after beginning the starvation experiment.

DIFFERENCE IN THE NEST-BUILDING ACTIVITY BETWEEN ISOLATED LEADERS AND ISOLATED FOLLOWERS

From a colony of *Camponotus* I selected one hundred medium-sized workers, I transferred these ants into a wooden arena and selected twelve most active leaders and twelve most inactive followers from the 100-ant group. Each of these twelve leaders and twelve followers was introduced into a working bottle, as described in my previous paper (Chen, 1937, Fig. 1), and allowed to work there for a period of 6 hours. During the period of work I recorded the reaction time (i.e., the time elapsed from the introduction

TABLE 10

DIFFERENCE IN SUSCEPTIBILITY TO STARVATION BETWEEN LEADERS AND FOLLOWERS

Date	Percentage Died		Percentage Living		Difference between Leaders and Followers
	Leaders	Followers	Leaders	Followers	
Sept. 4	0	0	100	100	0
5	0	0	100	100	0
6	6	0	94	100	6
7	15	4	85	96	11
9	34	6	66	94	28
11	40	8	60	92	32
13	47	9	53	91	38
15	50	9	50	91	41
17	57	9	43	91	48
19	61	9	39	91	52
21	74	12	26	88	62
23	78	12	22	88	66
25	83	12	17	88	71
27	83	14	17	86	69
29	85	15	15	85	70
Oct. 1	86	15	14	85	71
3	88	16	12	84	72
5	88	16	12	84	72
7	88	16	12	84	72
9	89	17	11	83	72
11	90	19	10	81	71
13	91	20	9	80	71
14	92	21	8	79	71

of the ant into the working bottle to the time when the ant began to build nest) for each ant; and at the end of the working period I collected the earth which the ants had excavated, and weighed it after it had been air-dried. From the records of the reaction time and the weight of the air-dried earth of the twelve leaders and twelve followers I calculated the averages, which are presented in Table 11.

The experiments as described above were repeated on September 2. The averages which I calculated from the data of the second experiment are also presented in Table 11.

A comparison of the mean reaction time and the mean weight of excavated earth of the leaders with those of the followers shows clearly that the reaction time of the leaders is much shorter than that of the followers, and that the mean weight of excavated earth (representing the amount of work done) of the leaders is more than that of the followers.

DIFFERENCE IN THE NEST-BUILDING ACTIVITY BETWEEN LEADER-GROUPS AND FOLLOWER-GROUPS

From a 100-ant group of healthy ants in a wooden arena I selected ten most active leaders and ten most inactive followers, I put the ten leaders together to form a small leader-group. Similarly, I put the ten followers together to form a small follower-group.

The group of ten leaders was put into a glass dish, which was inverted and placed in the center of the wooden arena (Fig. 2). The dish was then removed, and the ants were allowed to wander in the arena and to build a nest in any one or more of the corners of

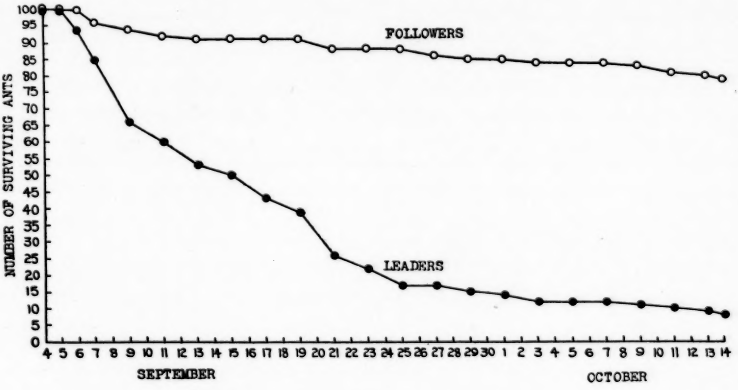

FIG. 5.—Susceptibility to starvation

TABLE 11

DIFFERENCE IN NEST-BUILDING ACTIVITY BETWEEN ISOLATED LEADERS AND ISOLATED FOLLOWERS

Trial No.	Kind of Workers	No. of Individuals	Mean Reaction Time (Minutes)	Mean Weight of Earth Excavated (Grams)
1	Leader	12	7.00	1.314
	Follower	12	199.25+	0.170
2	Leader	12	11.75	1.602
	Follower	12	54.50	1.086

the arena for a period of 2 hours. During this period the following records were taken: (1) the length of the interval from the time when the inverted dish was removed to the time when the ant or ants left the central plate to start their journey of exploration (this interval was called the "reaction time for leaving center," and was recorded for each ant); (2) the length of the interval from the time when the dish was removed to the time when the ant or ants began their nest-building work (this interval was called

the "reaction time for nest-building" and was also recorded for each ant). At the end of the working period of 2 hours the following records were taken: (1) the weight of the earth excavated by the ants; (2) the number of the nest-building places, i.e., the places inclosed by glass vessels buried in the corners of the arena, where ants had started nest-building work.

TABLE 12

DIFFERENCE IN REACTION TIME BETWEEN LEADER-GROUPS AND FOLLOWER-GROUPS

Trial No.	Reaction Time for Leaving Center (Minutes)				Reaction Time for Nest-building (Minutes)			
	Leader-Group		Follower-Group		Leader-Group		Follower-Group	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.....	2.0	1–6	3.6	1–7	5.0	3–7	24.0+	2–120+
2.....	1.0	1–1	1.5	1–2	6.4	1–14	18.8	15–25
3.....	1.3	1–2	11.5	1–22	5.1	3–12	120.0+	120+–120+
4.....	2.5	1–6	8.5	1–25	5.1	2–7	14.1	7–34
5.....	1.4	1–3	43.0	1–104	2.3	1–4	48.1	6–108
Av....	1.64	1.0–3.6	13.62	1–32	4.78	2.0–8.8	45.0+	30.0+–81.4+

TABLE 13

DIFFERENCE IN WEIGHT OF EARTH EXCAVATED AND NUMBER OF PLACES WHERE WORK STARTED BETWEEN LEADER-GROUPS AND FOLLOWER-GROUPS

Trial No.	Weight of Earth Excavated (Grams)		No. of Places Where Work Started	
	Leader-Group	Follower-Group	Leader-Group	Follower-Group
1.....	3.915	2.925	4	1
2.....	7.085	2.911	2	1
3.....	7.270	0.000	1	0
4.....	8.754	4.460	3	1
5.....	8.577	2.957	2	1
Av.....	7.120	2.651	2.4	0.8

The small group of ten followers which I selected was also allowed to work (under the same laboratory conditions as above) in the wooden arena for 2 hours, and the various records mentioned above were also taken.

The whole experiment, including the selection of small groups of ten leaders or followers and the observation of the nest-building behavior of these two groups, was repeated five times. The records of these five experiments are summarized in Tables 12 and 13.

Table 12 shows the records of the reaction time for leaving the central plate and for the beginning of the nest-building work. It shows both the means and the ranges of variation, i.e., the minimum and the maximum, of the reaction times for each experiment, as well as the averages calculated from the means and ranges of all five experiments. When an ant failed to start the nest-building work within the 2-hour working period, its reaction time for nest-building was recorded as 120+ minutes. Thus, both the mean and the range of the group of followers of Trial No. 3 were recorded as 120+ in Table 12 because all the ten ants of this group failed to start their work of nest-building. The data in Table 12 show clearly that both the mean reaction time for leaving center and the mean reaction time for nest-building were shorter in the groups of leaders than in the groups of followers.

Table 13 shows the records of the weight of earth carried out of the nest by the ants and the numbers of the places where nest-building work had been started. The data in this table show that the amount of work done by the groups of leaders was much more than that accomplished by the groups of followers. The data also show that the groups of leaders worked in more nest-building places than the groups of followers. In the case of followers, the ten ants usually worked in one place. But in the case of leaders, the ten ants usually worked in two or more places. The number of the places where the ants worked might be regarded as indicating the strength of cohesion of the group; the greater the number of the places where the ants started work, the less is the strength of cohesion of the group. Hence, we may say that the strength of cohesion of the groups of leaders was less than that of the groups of followers.

The results which I obtained concerning the differences between the nest-building behavior of the groups of leaders and that of the groups of followers may be summarized as follows: (1) The reaction time for leaving center was shorter in the groups of leaders than in the groups of followers. (2) The reaction time for nest-building was also shorter in the groups of leaders than in the groups of the followers. (3) The amount of work done by the groups of leaders was more than that done by the groups of followers. (4) The strength of cohesion of the groups of leaders was less than that of the groups of followers.

REVIEW OF LITERATURE AND DISCUSSION

The behavior of ants in nest-building has been studied both in the field and in the laboratory by many entomologists, and the results of these investigations have been described in detail by Forel (1928), Wheeler (1910), and others. In many respects the results of my observations and experiments agree with those of earlier workers.

According to Buckingham (1911), who studied the division of labor in American species of *Camponotus*, the small and medium-sized workers are, in general, much more active in nest-building than the large ones. My results confirm his observation, but, in addition, show clearly that the small workers are in general more active than the medium-sized ones, while the latter are in general more active than the large workers.

It is generally known that in human society there is a dominance-submission relationship. Among the animals, Schjelderup-Ebbe (1922, 1931) observed the "pecking order" in the domestic fowls, wild ducks, and many other birds. This is not an order of leadership in co-operative work but an order of tyrranny for individual privileges, superior ones being despotic over the inferior ones, and the latter timid toward the former. Similar despotic relationship has been observed in frogs (Schjelderupp-Ebbe, 1931) and

many other animals. But among the ants belonging to the same colony I have not observed such a relationship.

Fischel (1927) observed that among the hens a regular "race" frequently occurs. During this race every animal strives to be the "apex-animal," i.e., the foremost. The group depends upon the apex-animal in the direction and speed of running; hence the apex-animal is the leader of the group at this time. But the leader is also dependent upon the group, because she returns when the group does not follow her. Such a leader exists even in a group of two hens. Leadership is, however, only temporary in any one individual. It is usual that one hen leads the group for a while and is then replaced by another one. It is exceptional that one and the same hen remains on the "apex" of the group for a long time. The leader of the group is not the greatest tyrant of the "pecking order" of Schjelderup-Ebbe.

In some respects the leader in a group of ants engaged in nest-building is similar to the apex-animal in a group of hens. Both the apex-animal among the hens and the leader among the ants are temporarily the most active members of the group. The apex-animal leads the group in the direction and speed of running; the leading ant leads the group in the location and speed of nest-building. In both the hens and the ants, the position of leadership is occupied only temporarily by any one individual in the group. But, in other respects, the leader of ants is not similar to the apex-animal of hens. In a group of hens, the apex-animal returns when the group does not follow her. But in ants, the activity of the leader is sometimes less dependent on the group. It is occasionally observed that a single ant is working persistently in a favorable nest-building place while many others are resting or wandering, without following the example of the leader and participating in the nest-building work. In hens, it is only in an exceptional case that one and the same hen remains on the "apex" of a group for a long time. But in ants, when a group is formed by a very active and a very inactive individual, the former may remain as leader of the group for many days. The difference between the observations in hens and those in ants, however, may be partly or entirely due to the fact that the studies in hens made by Fischel were not carried out in the same way as my works in ants.

In regard to the difference in nest-building behavior between the leaders and the followers, it is not strange that the leaders have shorter reaction time and accomplish more work during a fixed experimental period than the followers, both in the isolated condition and in groups. However, it is rather unexpected that the strength of cohesion of a group of leaders is weaker than that of a group of followers. It is perhaps due to the same independent spirit and the great explorative activity of leaders that there is usually more diversity of opinions and less strength of cohesion in a human society when there is a greater than ordinary number of leaders.

SUMMARY

1. The author studied the social behavior of a common Asiatic variety of ants, *Camponotus japonicus* var. *aterrimus* Emery, and observed that, among the workers, there is a gradient in the activity in nest-building. The comparatively more active ant in a group initiated the co-operative building work and became the temporary leader of the group while others became the followers.

2. The leader-follower relationship was observed even in two-ant groups. When the difference in the activity of two ants was small, the leader-follower order was constant

only during a short period of a few hours. By summarizing the results of numerous determinations of the leader-follower order of any two ants in a small group of ants, the author observed a linear order of leader-follower relationship. This linear order, however, remained without change only for a short time and was found to be continuously changing in successive daily trials.

3. When the difference in activity between two ants was large, such as between the most active leader and the most inactive follower of a large group of ants, the leader-follower order remained constant for many days in the majority of the cases which the author studied.

4. The small workers of *Camponotus japonicus* are in general more active than the medium-sized workers; the latter, in general more active than the large workers.

5. The leaders in ants are more susceptible to chloroform, ether, drying, and starvation than the followers.

6. In nest-building, the mean reaction time of isolated leaders is shorter than that of the followers; the mean weight of excavated earth of the former is more than that of the latter.

7. There are many differences in the nest-building behavior between a leader-group and a follower-group: The reaction time of the former is shorter than the latter; the amount of work done by the former is more than that of the latter. The leader-group is more scattered and works in more nest-building places than the follower-group. The last difference shows that the strength of cohesion of the group of leaders is less than that of the group of followers.

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DIFFERENTIAL EFFECTS OF THE OVARIAN HORMONES ON THE TERRITORIAL REACTION TIME OF FEMALE ANOLIS CAROLINENSIS[1]

(One figure)

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MOST of the gradually increasing number of studies on the experimental effects of hormones in reptiles are histological, describing cellular changes resulting from extirpation of glands or of injections or implantations of hormone-containing substances. Such are the papers of Cunningham and Smart (1934), Evans (1935*a*, 1935*b*, 1935*c*), Forbes (1934), Hellbaum (1936), Herlant (1933), Houssay (1931), Mellish (1936), Padoa (1933*a*, 1933*b*), Regamey (1935), Schaefer (1933), Siler (1937), Takewaki and Fukuda (1935*a*, 1935*b*), and Turner (1935).

Pituitary and pregnancy-urine extracts induced winter-mating behavior in *Anolis carolinensis*, while ovariectomy produced changes in territorial behavior (Evans 1935*d*, 1936*a*, 1936*b*, 1936*c*). The present study is the result of an attempt to confirm previous work on the female and at the same time to improve the technique used[2] to measure the reaction time, a convenient method of discovering the effects that hormones have upon behavior.

Experiments were begun on November 27, 1935, and were terminated on June 11, 1936. Fourteen healthy adult females of *A. carolinensis* (weight, between 2.5 and 3.3 gm.) were selected to be used as resident lizards in the experiments. One of these (BD) had been used as a control in previous work (Evans, 1936*c*). The other thirteen were chosen from a group which had been sent from New Orleans a few weeks previously. A varying number of other females and young males (none weighed more than 3.3 gm.) were used as nonresidents to measure the reaction time of the selected fourteen resident females.

[1] The author is pleased to acknowledge the assistance given him by Professor A. B. Dawson, of Harvard University. The problem was suggested by Professor C. Murchison, of Clark University. Mr. G. Noreen and Mr. N. Pederson of Montana State University aided in the compilation of the data and preparation of Fig. 1.

[2] The resident female A (one that has been confined in one cage for several days) is watched carefully while a nonresident or strange female B is placed quietly in her cage. After a certain interval, which may vary from a few seconds to several minutes, female A challenges female B by flattening the sides of her body and by extending the small dewlap or fan which lies immediately beneath her lower jaw and which is actuated by muscles attached to a pair of slender bones which form part of the hyoid apparatus. This interval (measured with a stop watch) is termed the reaction time and can be readily observed at a distance of 15 or 20 feet with 6×-prism binoculars. Not until after the reaction time of each resident female in the laboratory has been tested are the nonresident lizards removed from the cages to be used again 24 or 48 hours later. It is important to retain as many nonresident lizards as there are resident lizards and each time to use any one nonresident in the cage of a different resident. This reduces to a minimum the part which the resident's memory of any particular nonresident lizard might play in determining her reaction time. If a resident female fails to respond or if her reaction time exceeds 13 minutes, a nonresponse is recorded.

The fourteen selected animals were isolated, each in a separate cage, and were handled only once weekly, when each received a few drops of cod-liver oil and orange juice. They were watered and fed daily and were irradiated with ultra-violet rays as described in detail in previous papers.

Prior to this study it was learned that a 13-month interval between the date of castration and the initial date of observations (Evans, 1936*c*) produced such a marked effect that the reaction time of spayed females averaged 1 minute, while that of the controls averaged 6 minutes. For the present study, however, the time at my disposal was not sufficient to continue observations for so long a period after spaying the females.

A fortunate circumstance presented itself in this connection. During the interval between November 27 and February 28 it was noted that the fourteen females being studied arranged themselves automatically into two distinct groups in regard to reaction time. Group A is comprised of six females whose average reaction time for this period was 3.633 minutes. Group B is comprised of eight females whose average for the same period was 1.689 minutes. Because previous work had shown that the reaction time is reduced following ovariectomy, it seemed likely that Group A with its intrinsically longer reaction time would probably respond to spaying more markedly than would Group B. At least, what change might occur would perhaps be easier to detect and record. Accordingly it was decided to spay Group A on February 28 and to continue B as the control group until June 11. Autopsy of the females of Group A on June 11 revealed a complete absence of ovarian tissue. The environments of both groups were identical so far as size of cages, food, water, humidity, temperature, and sunlight were concerned.

RESULTS

A total of 373 encounters was observed. Of this number, 202 pertained to the spayed Group A, while 171 pertained to the control Group B. The number of actual combats in which the resident female challenged with dewlap and then attacked the nonresident was 175 for Group A and 152 for Group B. Twenty-seven nonresponses on the part of the resident were recorded for Group A, while nineteen nonresponses were observed for Group B.

Three distinct periods of activity were noted in connection with Group A from November to June. The first period, which extended from November 27 to February 28, was the prespayed interval, marked by a relatively long average reaction time. The second period extended from the date of castration, February 28, to May 13, during which the reaction time was gradually reduced. In the third period (May 13 to June 11) the reaction time remained, rather uniformly, at a fraction of a minute. To compare Group A with the control Group B, the activity of the latter group was studied during the same periods which were adopted for Group A. In Group A there were 45 actual combats during the first period, 40 combats during the second period, and 90 combats during the third period. In Group B for the same periods of time there were 49, 45, and 58 combats, respectively.

For Group A the average reaction time of the females in the first period was 3.633 minutes. In the second period the average reaction time dropped to 1.602 minutes, and in the third period to 0.555 minutes. The average reaction time for Group B for the same three periods were, respectively, 1.689, 1.578, and 1.649 minutes.

Of the twenty-seven nonresponse cases of Group A, sixteen occurred before February

28, eight in the interval from February 28 to May 13, and three between May 13 and June 11. For the same periods the nineteen cases of nonresponses occurring in Group B were, six, seven, and six, respectively.

Table 1 gives a detailed account of the numbers of combats, nonresponses, and the average reaction time of each female of Group A (castrates) and Group B (normals) dur-

TABLE 1

Name	Number of Combats				Average Reaction Time (Minutes)			Nonresponses			
	Before 2/28	Between 2/28 and 5/13	5/13 to 6/11	Total	Before 2/28	Between 2/28 and 5/13	5/13 to 6/11	Before 2/28	Between 2/28 and 5/13	5/13 to 6/11	Total
	Group A (Castrates)										
BD	22	6	18	46	3.261	2.096	0.310	2	0	0	2
DN	4	9	14	27	3.830	2.210	0.600	8	1	0	9
DS	7	5	13	25	2.750	0.540	0.450	0	3	0	3
DK	1	4	18	23	2.000	1.670	0.280	2	3	0	5
DF	3	10	13	25	7.000	2.420	0.900	3	0	3	6
DO	8	7	14	29	2.960	0.680	0.790	1	1	0	2
Total	45	40	90	175	.....	.....	.....	16	8	3	27
Average	.....	.....	.....	.....	3.633	1.602	0.555	.....	.....	.....	.....
	Group B (Normals)										
DM	11	4	11	26	2.045	1.354	0.969	1	0	1	2
FX	0	4	11	15	0.000	2.187	0.979	0	3	0	3
DTT	7	7	11	25	1.071	0.857	3.423	0	0	2	2
FF	2	4	3	9	1.750	2.145	1.113	0	4	0	4
DR	9	10	4	23	0.777	1.366	0.588	2	0	0	2
DP	10	5	2	17	1.400	1.650	1.375	1	0	0	1
DW	2	5	7	14	2.000	1.550	1.691	2	0	1	3
DQ	7	7	9	23	2.785	1.513	3.055	0	0	2	2
Total	49	45	58	152	.....	.....	.....	6	7	6	19
Average	.....	.....	.....	.....	1.689	1.578	1.649	.....	.....	.....	.....

ing the periods mentioned above. Figure 1 shows graphically the erratic and unpredictable behavior of both groups up to February 28, with a subsequent gradual but marked change in the behavior of the castrates, but not of the normals, after February 28. In Table 2 is given the reaction time of each encounter recorded for a typical normal resident female (DQ) and for a typical spayed resident female (BD). The records of these two females are quite representative of the two groups studied. Lack of space prevents publication of the reaction time of each observed encounter of each of the fourteen females.

It is interesting to note the change in female behavior after the ovaries are removed. Whereas the normal resident female not only watches very closely the strange lizard being placed in her cage but also shifts her gaze to me, studying every movement which I make, the spayed female after a period of 2½ months following ovariectomy pays almost no attention to me but concentrates only on the strange female entering her cage. In

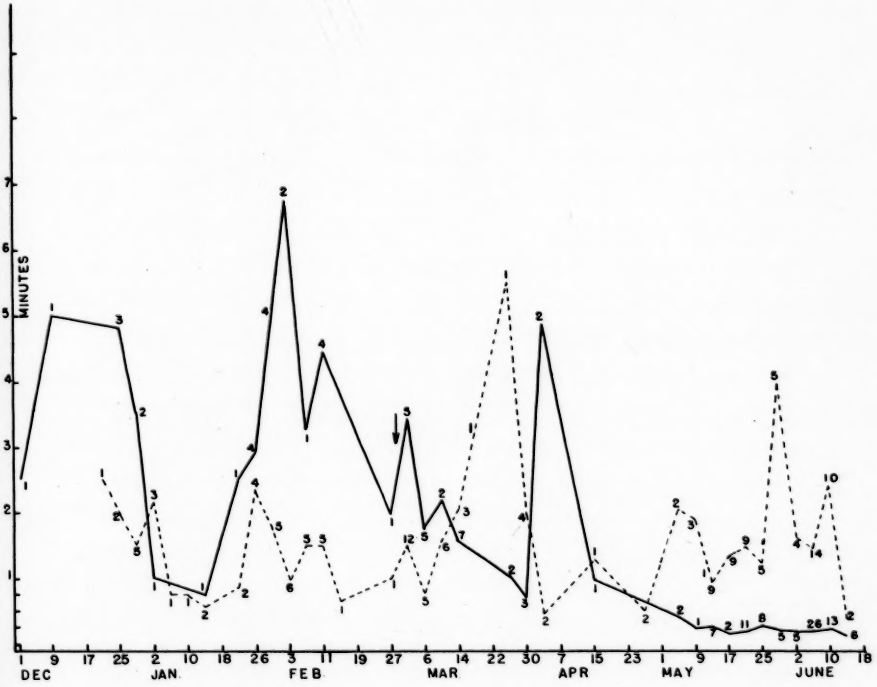

FIG. 1.—Graph showing difference in reaction time between the females of Group A (spayed on February 28 as indicated by arrow) and those of Group B (normal), between the end of November, 1935, and June, 1936. Heavy line indicates Group A; light broken line indicates Group B. Numerals on the graph indicate the number of encounters in any 4-day interval (horizontal scale) averaged to yield the reaction time in minutes (vertical scale). Separate dots indicate single encounters of extraordinary length which were recorded by Group A and not averaged to appear on the heavy line of Group A.

over 90 per cent of the observations she shows no hesitancy but challenges the intruder in characteristic fashion immediately after the cage door is closed or sometimes even before. Figure 1 indicates that the spayed female, after 2 months or more, reacts, quite consistently, in 15 seconds or less. It seems reasonable to infer that the last remnant of ovarian hormones in the blood has been exhausted after such a period of complete ovariectomy. Table 3 gives a series of fourteen detailed observations; seven pertaining to a typical normal female (DQ) and seven relating to a typical spayed female (BD). These

notes were selected at random from the daily records taken during the three periods mentioned above, from November to June. They emphasize the slow but unmistakable change which occurs in the territorial behavior of a female after castration in contrast to the lack of change observed in the behavior of a typical normal female.[3]

TABLE 2

REACTION TIME IN MINUTES OF TWO FEMALES FROM NOVEMBER TO JUNE

Date.....	.....	11/27	12/29	1/1	1/26	1/29	2/1
BD.....	(2.88)*	2.5	6.0	.....	1.75	11.0	N†
DQ.....	.....	.....	3.0	3.0	4.00	5.0	1.5
Date.....	2/6	2/8	2/28	2/29	3/1	3/4	3/8
BD.....	N	1.5	‡	.....	3.5	2.00	3.5
DQ.....	1.5	1.0	1.5	2.0	.....	1.75	3.0
Date.....	4/24	5/6	5/11	5/14	5/16	5/17	5/19
BD.....	3.00	0.33	0.25	1.5	1.0	0.125	0.20
DQ.....	0.59	1.00	0.75	2.0	.....	2.000	N
Date.....	5/20	5/21	5/22	5/26	5/31	6/2	6/3
BD.....	0.33	0.125	0.50	0.33	0.34	0.25	0.125
DQ.....	1.33	0.750	.....	1.00	N	.....	5.500
Date.....	6/4	6/5	6/6	6/8	6/9	6/10	6/11
BD.....	0.11	0.07	0.08	0.05	0.33	0.08	0.04
DQ.....	.....	1.59	3.50	9.84	.....	.....	.....

* 2.88 is average reaction time in minutes of 17 encounters of 1 D between July and November, 1935.
† N, Nonresponse
‡ Date on which BD was spayed.

CONCLUSIONS

Three hundred and seventy-three observations recorded in connection with the present paper, as well as 161 observations recorded previously (Evans, 1936*c*), indicate that the territorial response of a normal female is quite erratic. She seems to respond differently on successive occasions to a similar environmental stimulus, which is reflected in the record of her reaction time. It is suggested that the higher brain centers are active in controlling her responses, with the result that she shows a greater degree of caution, which increases her reaction time and at the same time makes it impossible to predict even approximately what her reaction-time response to the intruding lizard will be on a particular occasion. Since the ovaries are intact in the normal female, it is probable that it is the ovarian hormones which increase the degree of inhibition of the higher brain centers. In a natural habitat too much boldness in attacking intruders might bring bodily danger to a resident female.

With the slow elimination of ovarian hormones from the body after ovariectomy, the territorial response of the female alters gradually from the highly erratic and somewhat

[3] Recent field studies of *Anolis* at the Harvard Arboretum, Soledad, Cuba (Evans, 1938), confirm previous work on this genus which had been carried out in the laboratory and leave no doubts regarding the territorial responses of males and females where they are given opportunity to acquire territory.

TABLE 3

CASE RECORDS OF BD (SPAYED ON FEBRUARY 28) AND DQ (CONTROL) SELECTED TO EMPHASIZE CHANGE IN BEHAVIOR OF BD

DQ	BD
1/29/36. Sun shining Female placed in cage of female DQ at 3:09 P.M. At 3:14 DQ challenged with dewlap display and flattened sides. DQ challenged NR* female, chasing her down from top of cage twice by 3:18.	2/8/36. Sun shining Female placed in cage of BD at 3:48½ P.M. BD challenged vigorously at 3:50.
3/4/36. Sun shining Female placed in cage of DQ at 11:05½ A.M. At 11:07¼ DQ challenged.	3/4/36. Sun shining Female placed in cage of BD at 11:32 A.M. At 11:34 BD challenged. NR female bobbed head in reply. At 11:36 BD chased her.
4/24/36. Sun shining Female placed in cage of DQ at 3:05 P.M. Thirty-five seconds later DQ challenged NR female.	4/24/36. Sun shining Female placed in cage of BD at 2:55. At 2:58 BD stood rigid and challenged NR female. From 3:00 to 3:10 she chased NR female about the cage and challenged repeatedly.
5/6/36. Sun shining Female placed in cage of DQ at 5:09. At 5:10 challenged NR female who bobbed in reply 30 seconds later. DQ then chased NR female.	5/6/36. Sun shining Female placed in cage of BD at 5:37. Twenty seconds later BD challenged and immediately chased NR female up and down the pieces of bark in the cage. This, in spite of the fact that BD had been disturbed only 5 minutes before.
5/17/36. Sun shining Female placed in cage of DQ at 5:27. At 5:29 DQ challenged NR female but did not chase her.	5/17/36. Sun shining Female BD on screen of door. NR female placed on screen at 5:00 P.M. BD challenged in 8 seconds (after I closed the door) and chased NR, while I stood 18 inches away. NR female bobbed reply once.
6/3/36. Sun shining Female placed in cage of DQ at 4:57. At 5:02½ DQ came down a branch and challenged NR female, then chased her.	6/3/36. Sun shining Female placed in cage of BD at 4:50. Seven seconds later BD dashed at NR female, bit her, then, as NR withdrew, BD challenged and chased her about the cage. BD ignored me in her absorbed interest in NR female.
6/6/36. Sun shining Small male placed in cage of DQ at 4:36. At 4:39½ DQ challenged NR male briefly. The male ran away.	6/6/36. Sun shining Small male placed in cage of BD at 4:25. Five seconds later BD challenged NR male and continued to do so for 2 minutes. Then she charged and bit. Then nudged him with her nose. Finally, she chased him vigorously.

* Nonresident.

inhibitory behavior, which is characteristic of the normal female, to the more or less stereotyped, predictable, and almost instantaneous response typical of the spayed female. It is probable that the higher brain centers in the spayed female are acting without the inhibitory effect of the hormones of the ovaries.

Observations indicate that lizards which exhibit a relatively long reaction time are also prone to show a larger number of nonresponses as compared with lizards having relatively short reaction time. This is borne out in the present study, since sixteen nonresponses are recorded for Group A from November 27 to February 28, and the reaction time for the period was 3.633 minutes. In contrast, six nonresponses are recorded for Group B between November 27 and February 28, and the reaction time was 1.689 minutes for the same period.

The effect which ovariectomy has upon the number of nonresponses in Group A is striking. Out of the total of twenty-seven cases, sixteen occurred before the ovaries were removed, eight were registered between February 28 and May 13 and only three after May 13. No appreciable change occurred in the number of nonresponses in Group B for these three periods as shown by the fact that there were six in the first period, seven in the second period, and six in the third period (after May 13).

The profound effect which spaying has upon the reaction time of female lizards is shown by the fact that for Group A the average reaction time before castration was 3.633 minutes. This was almost two and one-half times as long as that of Group B, which was 1.689 minutes. The average reaction time for Group A during the third period (from May 13 to June 11) was reduced to 0.555 minutes. This was only one-third the reaction time of Group B (which was 1.649 min.) for the same period.

SUMMARY

During three periods—(1) November 27, 1935, to February 28, 1936; (2) February 28 to May 13; and (3) May 13 to June 11—373 separate observations were made on two groups of adult females of the lizard, *A. carolinensis*.

Group A comprised six females which were spayed on February 28. Group B comprised eight unoperated females which served as controls. The average reaction time (the time required for an isolated resident lizard to respond overtly to a nonresident lizard placed in its cage) of Group A in the first, second, and third periods was 3.633, 1.602, and 0.555 minutes, respectively. The average reaction time of Group B in the same three periods was 1.689, 1.578, and 1.649 minutes, respectively.

Group A showed sixteen nonresponses in the first period, eight in the second period, and three in the third. Group B showed six, seven, and six nonresponses in the first, second, and third periods, respectively.

It is concluded that ovariectomy profoundly alters the territorial response of the female lizard by removing the natural inhibiting effect which the hormones of the ovaries have upon behavior.

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A CORRELATION OF RESULTS ON OXYGEN CONSUMPTION OBTAINED BY THE WINKLER METHOD AND BY RESPIROMETERS, USING AS A STANDARD THE METHODS OF VAN SLYKE

(One figure)

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A VARIETY of methods and apparatus is employed in the measurement of oxygen consumption by small animals. The method of Winkler, or one of its modifications, has been much used for the determination of dissolved oxygen, while respiration in air has been commonly measured by some one of the manometric methods (Dixon, 1934). To our knowledge, however, no correlation has been made of the relative accuracy under experimental conditions of these two types of methods. A paper will shortly appear from this laboratory by Dr. Gertrude Evans, discussing the oxygen consumption of various species of salamanders in air and water. In this work dissolved oxygen was measured by Winkler's method as modified by Rideal and Stewart (1901); the manometric respirometers were constructed on the principle of Harrington and Crocker's (1923) apparatus. Without direct tests, it was impossible to know whether the results obtained by one method were comparable to those obtained by the other. For this reason and the need of comparison of the relative accuracy of different methods, it was suggested to the writer by Dr. W. C. Allee that such a correlation be made, using as a standard the Van Slyke methods applicable both to dissolved oxygen (Van Slyke and Neill, 1924; Oesting, 1934) and to air analysis (Van Slyke and Sendroy, 1932).[1]

The plethedontid salamanders used in the experiments of Dr. Evans were a convenient form for this study, since they are able to utilize both dissolved and atmospheric oxygen. Preliminary to that paper a few notes on the species differences in oxygen consumption between the forms (*Eurycea bislineata bislineata*, *Desmognathus fuscus fuscus*, *D. fuscus ochrophaeus*, and *Gyrinophilus porphyriticus*)[1] are included in the last section of this report.

All analyses of dissolved oxygen were made on water which had no less than 76.4 per cent saturation (mean, 89.1) at the end of the test period. In the air determinations the lowest value of oxygen obtained was 18.4 per cent (mean, 19.8); this is 95.6 per cent of the oxygen content of the outdoor air, which was found to be 20.69 per cent. Therefore, these results do not deal with low oxygen concentrations. Furthermore, in the water experiments glass-stoppered bottles were used which eliminated the question of the efficacy of the seal.

The results are calculated to 0° C. and 760 mm. mercury pressure, and the amount of oxygen removed by respiration is corrected to the volume of the containers; therefore, the figures show actual amounts of oxygen consumed at standard temperature and pressure. Some confusion arises in the literature from the failure of workers to report whether

[1] We are grateful to Dr. Allee for continued advice and criticism during the course of the work; we wish also to express our thanks to Mr. K. P. Schmidt of the Field Museum for the identification of the animals.

their results are calculated on the basis of standard conditions or room conditions, and whether the consumption is per liter or corrected to volume.

In the experiments on the utilization of dissolved oxygen, two samples were drawn from each test bottle at the end of the 5-hour test period. One of the samples was then analyzed by the Winkler method, the other by the Van Slyke. Likewise in the experiments on aerial respiration, the sample to be analyzed by the Van Slyke method was taken from the respirometer. Therefore, each paired comparison of the two methods is for the same animal, over the same test period.

COMPARISON OF THE RESULTS OBTAINED BY THE WINKLER AND VAN SLYKE METHODS OF MEASURING DISSOLVED OXYGEN

Nine experiments with *D. fuscus ochrophaeus* and four with *E. bislineata bislineata* provided sixty-four paired comparisons of oxygen content at the end of the test period and a similar number of consumption records.

Method.—Each of the thirteen experiments consisted of a 5-hour test period for six animals. Eight glass-stoppered, narrow-necked bottles with a capacity of 250 cc. were used, two of which were blank controls, and six contained animals. At the beginning of each experiment the bottles were filled with the synthetic pond water described by Allee, Bowen, Welty, and Oesting (1934). However, in Experiments XII and XIII lake water, free of chlorine, was used. No difference in result was detected despite the higher oxygen content of the latter (8.472 cc. oxygen per liter, as compared to 6.140, average "blank" values).

At the conclusion of the 5-hour period, two samples were drawn, one of about 14 cc. for the Van Slyke method, the other, for the Winkler with a mean volume of 117.3 cc. Sampling was most satisfactorily accomplished by a Y siphon tube, one arm of the Y was smaller in bore than the other; this permitted both the large and the small sample to be drawn simultaneously. The analyses were conducted according to the directions for the permanganate modification of the Winkler method given in "Standard Methods for the Examination of Water and Sewage" (1933); and according to the method of Van Slyke and Neill (1924), modified by Oesting (1934). A 5-cc. sample was used, and the measurement of carbon dioxide was omitted from the latter method.

For absolute results the standardization of the thiosulphate is of great importance. After using 0.01 N potassium biniodate for the first nine experiments and finding it unsatisfactory, a change was made to potassium bichromate[2] following the procedure of Willard and Furman (1933). Sodium thiosulphate of approximately 0.1 N was standardized against potassium bichromate of that strength, then diluted by customary quantitative methods to 0.01 N, at which concentration the samples were titrated. The standardization is more accurate with the stronger solutions. All experiments prior to Experiment X have been recalculated to the standardization value of the thiosulphate obtained by the bichromate method.

Results.—The reliability of each method depends upon the extent of error within the method itself and upon the competence of the operator. Tests of reliability consisted of analysis of a number of blank samples of the same water on the same day. Nine samples analyzed by the Winkler method gave a mean value of 5.243 cc. oxygen per liter with a standard deviation of 0.026 and a coefficient of variation of 0.50 per cent. By the Van

[2] We are indebted to Dr. W. C. Pierce of the chemistry department for this suggestion.

Slyke method a mean value of 5.286 cc. oxygen per liter was obtained from ten samples. The standard deviation in the latter case was 0.074, and the coefficient of variation 1.40 per cent. This larger variation in the Van Slyke analyses was due to the deviation of one sample (5.468 cc.).

Twenty-three paired comparisons of one or two "blanks" in the thirteen experiments gave a mean value of 6.455 cc. oxygen per liter by the Winkler method and 6.433 cc. by the Van Slyke. The difference of 0.022 cc. is not statistically significant ($P = 0.4318$).[3] In Table 3 (A) will be found a summary of the results on oxygen content at the end of the test period. These figures are presented to denote the agreement between the two methods at the base level for calculation of oxygen consumption. The oxygen present at the start of the experiment, obtained from the analysis of the "blanks," has been omitted,

TABLE 1*

EXAMPLES OF SEPARATE ANALYSES IN THE WINKLER–VAN SLYKE COMPARISONS

Species	Expt.	Animal	Cc.O_2/Liter, Final		Mm.$^3O_2$/Gm.-Hr. Consumed	
			Winkler	Van Slyke	Winkler	Van Slyke
Desmognathus fuscus ochrophaeus.....	I	1	4.67	4.75	35.6	33.1
	II	4	4.90	4.91	49.2	39.5
	IV	2	5.43	5.49	46.1	44.7
	V	5	5.78	5.61	32.6	45.0
	VIII	3	5.34	5.32	35.8	40.4
Eurycea bislineata bislineata.	X	1	4.52	4.40	70.1	74.6
	X	6	5.04	5.18	61.7	55.7
	XI	5	4.75	4.72	70.8	71.1
	XII	5	6.52	6.22	88.9	89.9
	XIII	3	6.59	6.69	72.5	73.0

* To assure random choice, the first and thereafter every ninth case in the *Desmognathus* experiments, and the first and every fourth in the *Eurycea* experiments, have been taken.

since it adds nothing further because variation between the results from the two methods is in the same range of magnitude (see above, $P = 0.4318$). The usually greater variability between the two methods in the values found for oxygen consumption (mm.^{3} per gram-hour) is explicable on the fact that this figure is dependent not only on the figures given in the tables (1 and 3) for oxygen present per liter at the end but also on the "blank" values.

Calculation of oxygen consumption gave a mean of 62.0 mm.^{3} per gram-hour and 62.5 mm.^{3} by the Winkler and Van Slyke methods, respectively, with P equal to 0.7342 (Table 3). Table 1 contains a number of randomly chosen comparisons to show some of the original figures on which the summary in Table 3 is based.

[3] Probability (P) indicates the chances of obtaining a like result from random sampling; $P = 0.05$, five chances in a hundred, is the upper limit of statistical significance. The smaller the value of P, the greater is the significance, and, therefore, a P of 0.4 is well above any significant difference. Probability has been calculated from "Student's" tables on the basis of paired comparisons.

COMPARISON OF THE RESULTS OBTAINED ON AERIAL RESPIRATION BY THE MANOMETRIC RESPIROMETERS AND THE METHOD OF VAN SLYKE

The oxygen consumption in air of *D. fuscus fuscus* and *E. bislineata bislineata* during 15 hours and of *G. porphyriticus* during 10–12 hours has been measured by stationary manometric respirometers and by the method of Van Slyke and Sendroy. A total of thirteen experiments provided twenty-nine paired comparisons of the results by the two methods.

Description of the respirometers.—Five respirometers (Fig. 1) were used—two as "blanks" to measure the effect of barometric change, the other three held salamanders. The apparatus is designed on the principle of Harrington and Crocker's (1923) for the determination of respiration in seeds. Three main modifications have been introduced to adapt it for animals, considering the greater size and motility:

1. The alkali, which is used to absorb carbon dioxide, is contained in a glass cup covered by copper screening (Fig. 1, *A*)
2. The respiratory chamber (*RC*) is larger, about 140 cc. capacity, and the animal is loose within it
3. A third inlet to the chamber (*II*) allows aeration of the apparatus at intervals in a long experiment. It is also essential for the removal of an uncontaminated air sample for Van Slyke analysis

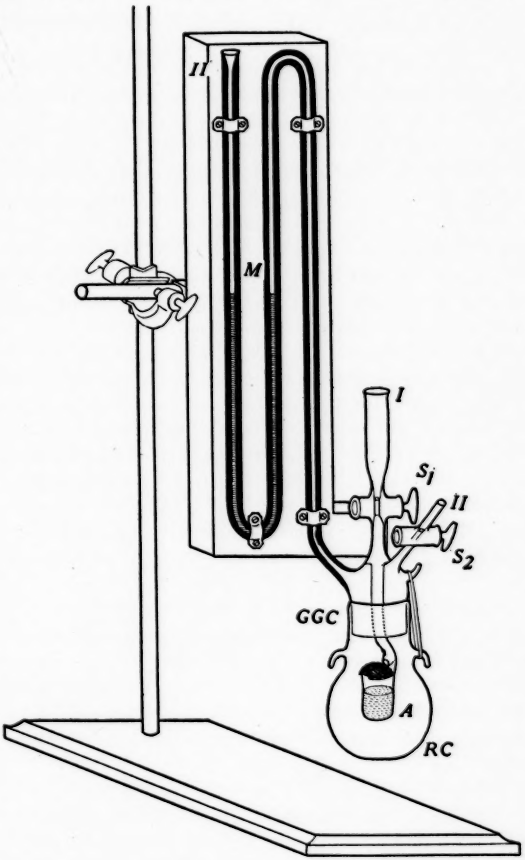

FIG. 1.—The respirometer used in these experiments, modeled on that of Harrington and Crocker. *I, II, III:* the three inlets; S_1 and S_2: stopcocks to inlets *I* and *II*, respectively; *A:* glass cup for alkali, covered by copper screening; *GGC:* ground glass-connection; *M:* water manometer; *RC:* respiratory chamber.

The following procedure was used. Six-tenths of a cubic centimeter of distilled water was run into the manometer (Fig. 1, *M*) through inlet *III*. This was sufficient to fill it halfway up the scale. Two cubic centimeters of 30 per cent KOH was poured into the alkali cup (*A*). In these experiments the alkali inlet (*I*) was not used for this, since it must

be dry for the later removal of the air sample. One cubic centimeter of tap water provided moisture for the animal in the chamber (*RC*). The animal was introduced, and the ground-glass connection (Fig. 1, *GGC*) closed. The five apparatuses so prepared, two without salamanders, were placed in the water bath, the level of which covered the ground-glass connections. They remained there with stopcocks S_1 and S_2 open for 1 or more hours. This allowed both temperature equilibration and a period of acclimation for the animals.

Before starting an experiment each apparatus was aerated by a stream of outdoor air. This had been collected over water the day previous and stored in a large carboy in the constant temperature room in which the experiments were conducted. After aeration the stopcocks were closed, and the capillaries in inlets *I* and *II* filled with mercury. Recording of the time, temperature, and barometric pressure completed the preparations. At the end of the allotted period the readings were repeated, and the deflection of the two levels of the water manometer noted. For the latter purpose a strip of co-ordinate paper was shellacked to the board behind the manometer, and a millimeter scale fastened beside the right arm of the manometer. The deflection due to barometric or temperature change was registered by the blank instruments, and this correction was made in the readings of the other respirometers.

Collecting the air samples.—The air samples for Van Slyke analysis were taken from the respirometers as quickly as possible by collecting them over mercury. The gas-sampling device, used also as a storage container, is figured by Van Slyke, Sendroy, and Liu (1932, p. 555).[4] It consists of a 35-cc. ovoid bulb open at both ends into short capillary tubes, one of which is closed by a three-way stopcock; the other connects by rubber tubing to a mercury reservoir. By lowering or raising the reservoir, air can be drawn in or expelled from the container. In using the sampler, a glass bridge of capillary tubing, fitted with a thick-walled rubber ring at the far end, was connected to the stopcock-closed capillary by nitrometer tubing. The rubber tip could be fitted tightly into the cup (Fig. 1, inlet *I*) of the respirometer for sampling, or into the cup of the Van Slyke apparatus during transfer of the sample for analysis.

As air was drawn from the respirometer over into the gas container by allowing the mercury level to fall, water flowed into the respirometer through inlet *II* to replace the volume of removed air. At no time was the air sample in contact with outside air. At the time of analysis the air sample was transferred to the Van Slyke by the reversal of the same procedure. Analysis then proceeded according to the directions given by Van Slyke and Sendroy (1932).

Results.—Analysis of nine samples of laboratory air to test technical accuracy by the Van Slyke method gave a mean value of 20.672 per cent oxygen content, with a standard deviation of 0.092, and a coefficient of variation of 0.44 per cent. The respirometers do not give absolute results but, by deflection of the manometer levels, measure a barometric or temperature change outside the instrument, or a change in pressure within owing to removal of oxygen in respiration.

Part B of Table 3 summarizes the measurements of oxygen consumption by the two methods. As found in the Winkler-Van Slyke comparison, no significant difference exists in the results. The total mean value was 41.8 mm.^{3} oxygen per gram-hour by the

[4] The use of this apparatus was suggested to the writer by Dr. M. Hanke of the local department of biochemistry.

respirometers and 43.0 mm.³ by the method of Van Slyke ($P = 0.4542$). Table 2 presents a random selection of separate analyses.

Of the four methods of measuring oxygen consumption discussed here, those of Van Slyke and his associates, designed primarily for the analysis of blood gases, are the more time-consuming and require the more practiced technician. However, when the method is applied to dissolved oxygen, a small sample, 5 cc. or less, may be utilized. Furthermore, it is free from the errors which may be introduced into the results of the Winkler method, at least when unmodified, by nitrites, iron compounds, and the absorption of

TABLE 2*

EXAMPLES OF SINGLE ANALYSES IN THE RESPIROMETER–VAN SLYKE COMPARISON

Species	Individual	Respirometer	Van Slyke
E. bislineata bislineata...	C	103.0	106.8
	F	78.1	75.0
	C	50.7	51.8
	C	52.5	61.0
	F	55.0	53.9
	E	58.1	65.9
D. fuscus fuscus.....	E	39.3	36.2
	C	43.8	43.1
	F	46.5	46.1
	E	38.2	35.4
	C	45.4	44.9
G. porphyriticus.....	C	10.8	14.5
	C	12.3	12.2
	C	14.9	19.2
	F	23.3	24.5

* To assure random choice, one-half of the cases are given, every alternate one being taken. Results in mm.³ O_2 /gm.-hr.

iodine (Allee and Oesting, 1934). Air analysis by the Van Slyke method involves the collection and storage of air samples. This is more difficult than the collection of uncontaminated water samples. The method of Van Slyke has, however, the advantage of a sharp distinction between the pressures exerted by oxygen, carbon dioxide, and nitrogen, which may all be measured in one sample. It might, therefore, be of use in finding whether the absorption of carbon dioxide by alkali in respirometers was complete.

In general, the results indicate that the permanganate modification of the Winkler method for determining dissolved oxygen and manometric respirometers designed on the principle of Harrington and Crocker's apparatus for aerial respiration, as used here, are fully adequate in biological studies.

THE OXYGEN CONSUMPTION OF THE VARIOUS SPECIES

Although this report covers work which was begun as a technical problem only, the use of different species to introduce experimental variation brought out certain facts which are interesting because they are related to species differences. In Table 4 the species are listed in order of body weight, and the mean oxygen consumption for each is

recorded, in water (A) and in air (B). The oxygen values are averages of the results obtained by the two methods which were being compared.

Each species has a markedly different rate of oxygen consumption when tested in the same medium. For example, in water *D. fuscus ochrophaeus* consumed 47.5 mm.^{3} oxygen per gram-hour while *E. bislineata* averaged as much as 76.7. This difference is entirely

TABLE 3

SUMMARY OF MEASUREMENTS ON FINAL OXYGEN CONTENT AND CONSUMPTION IN WATER BY THE METHODS OF WINKLER AND VAN SLYKE (A); AND OYXGEN CONSUMPTION IN AIR BY THE METHOD OF VAN SLYKE AND BY MANOMETRIC RESPIROMETERS (B)

A. Comparison of the Results Obtained by the Methods of Winkler and Van Slyke

Species	No. Expts.	No. Comparisons	Winkler	Van Slyke	*P*
			Final Cc. Oxygen per Liter		
D. fuscus ochrophaeus	9	46	5.264	5.240	0.2751
E. bislineata bislineata	4	18	5.715	5.748	0.2683
Total	13	64	5.490	5.494	0.9204
			Mm.^{3} Oxygen per Gram-Hour Consumed		
D. fuscus ochrophaeus	9	46	46.0	49.2	0.1288
E. bislineata bislineata	4	18	78.0	75.8	0.0896
Total	13	64	62.0	62.5	0.7342

B. Comparison of the Results by Respirometers and the Method of Van Slyke

Species	No. Expts.	No. Comparisons	Mm.^{3} Oxygen per Gram-Hour Consumed		
			Respirometer	Van Slyke	*P*
D. fuscus fuscus	4	10	41.2	41.0	0.8694
E. bislineata bislineata	4	11	67.9	69.9	0.1728
G. porphyriticus	5	8	16.4	18.2	0.0973
Total	13	29	41.8	43.0	0.4542

significant ($P < 0.0001$). The three forms tested in air likewise have differences in rate of consumption which are completely significant. Here it will be seen (Table 4, B) that the rate is inversely proportional to body weight, the lightest animal, *E. bislineata*, consuming 68.9 mm.^{3} per gram-hour, and the heaviest, *G. porphyriticus*, only 17.3, with *D. fuscus fuscus* intermediate in both respects. This observation is in accord with the fact that the rate of respiration is commonly inversely proportional to mass. *E. bislineata*, however, appears to have a rate of consumption independent of this factor to some extent, indi-

cated in its greater rate as compared to that of the lighter *ochrophaeus* variety of *D. fuscus*. It has been noted that *E. bislineata* is a more active animal.

The same individuals of *E. bislineata* were used in the air and water experiments. The apparently lower respiration in air (Table 4) is not, however, statistically significant ($P = 0.1208$). Likewise, differences between the two varieties of *D. fuscus*, one used in water, the other in air, is not significant ($P = 0.1096$). These problems will be treated at further length in the subsequent paper.

TABLE 4*

MEAN OXYGEN CONSUMPTION OF THE THREE SPECIES OF PLETHEDONTID SALAMANDERS

Species	Average Weight in Grams	No. Determinations	No. Animals	$Mm.^3O_2$/Gm.-Hr. Consumed
A. Experiments in Water				
D. fuscus ochrophaeus.....	0.92	46	7	47.5
E. bislineata bislineata....	1.22	18	6	76.7
B. Experiments in Air				
E. bislineata bislineata....	1.13	11	3	68.9
D. fuscus fuscus.....	2.03	10	3	41.1
G. porphyriticus.....	10.35	8	3	17.3

* Experiments in water lasted 5 hours (A); those in air, 10–15 (B). Results are given in cubic millimeters of oxygen consumed per gram-hour.

SUMMARY

1. A comparison of the relative accuracy of methods of measuring oxygen consumption in air and water has been made, utilizing plethedontid salamanders.

2. The permanganate modification of the Winkler method for determining dissolved oxygen and manometric respirometers, similar to those of Harrington and Crocker, have been compared, using the method of Van Slyke and Neill and Van Slyke and Sendroy as a standard.

3. From sixty-four paired comparisons it has been found that the two methods of determining the amount of dissolved oxygen agree and that oxygen-consumption determinations based on the two methods of analysis have no statistical difference (62.0 and 62.5 $mm.^3$ oxygen per gram-hour consumed, mean total value) with a probability of 0.7342.

4. Likewise, 29 comparisons of aerial respiration measured by the respirometers and the method of Van Slyke give no significant difference (41.3 and 43.0 $mm.^3$ oxygen per gram-hour, $P = 0.4542$).

5. Incidental to this technical problem it is noteworthy that the three species of salamanders have a significantly different rate of oxygen consumption when tested in the same medium.

6. This difference appears in part to correlate with an inverse relationship between oxygen use and body weight. However, in *Eurycea bislineata* there seems to be a metabolic rate independent of this factor.

7. When rate of consumption in air and water is compared in *Eurycea bislineata* and the two varieties of *Desmognathus fuscus*, no statistical difference is found.

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THE ACTION OF ANESTHETICS ON AMOEBA PROTOPLASM[1]

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DURING the past few years Heilbrunn and his students have gradually evolved a theory which seeks to explain stimulation and anesthesia in terms of colloid chemical changes in the protoplasm (see Heilbrunn, 1928; Heilbrunn and Daugherty, 1933; Heilbrunn and Mazia, 1936; Angerer, 1936; Mazia and Clark, 1936). According to this theory, stimulation, whether electrical, mechanical, or radiational, causes a series of changes, the first of which is a release of calcium from the cell cortex. Such a calcium release may well initiate an increase in permeability, and may be responsible for the increases in permeability which are believed to follow stimulation. Once released from the cortex, the calcium enters the cell interior and causes there first a decreased viscosity of the protoplasm. Then as the concentration of free calcium in the interior increases, there follows a characteristic clotting reaction of a type which is believed to occur whenever protoplasm comes in contact with a sufficient quantity of free calcium. This clotting reaction, which is believed to be fundamentally similar to the surface-precipitation reaction which occurs whenever protoplasm is forced out of a cell into a medium containing free calcium (see Heilbrunn, 1928 [chap. xiii]), is thought to represent the essential response of protoplasm to stimulation.

On the basis of the foregoing theory, anesthetics which prevent response to stimulation should act in one of two ways. They should either prevent release of calcium or prevent the clotting reaction initiated by the released calcium. There is evidence that fat solvent anesthetics do not prevent the release of calcium (Mazia and Clark, 1936) but that they do prevent a surface precipitation reaction (Heilbrunn, 1934*a*).

The present paper is an attempt to test the Heilbrunn theory on amoebae. Earlier work has shown that by centrifugal tests on *Amoeba proteus* and *Amoeba dubia* it is possible to study the viscosity both of the cortical plasmagel and of the interior plasmasol (see Heilbrunn and Daugherty, 1931 and 1932). In studying anesthesia or narcosis in amoeba it must be remembered that these terms can be used to describe two somewhat different phenomena. In the first place, anesthetics may prevent amoeboid movement, or, second, they may prevent the normal response to external stimulation. When an amoeba is irradiated or subjected to a mechanical or electrical stimulus, it typically responds by rounding up. In the discussion that follows I shall be concerned chiefly with the observable effects of various concentrations of certain fat-solvent anesthetics on the viscosity of the exterior and interior protoplasm, and no great emphasis will be placed on the question as to whether or not a state of anesthesia or narcosis has been achieved.

Brinley (1928*a*) and Frederikse (1933) have studied the effect of fat-solvent anesthetics on the viscosity of amoeba protoplasm, using the method of Brownian move-

[1] A thesis in zoölogy presented to the faculty of the graduate school of the University of Pennsylvania in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The investigation was aided in part by a grant from the Committee on Radiation of the National Research Council administered by L. V. Heilbrunn.

ment. Their results are not absolutely in agreement with each other. Both obtained liquefaction and this in the case of Frederikse's experiment was followed by gelation. Frederikse studied the viscosity both of the granular protoplasm and of the outer apparently highly fluid ectoplasm (Mast's hyaline layer). He did not distinguish between plasmasol and plasmagel.[2]

MATERIALS AND METHOD

The amoebae (*A. dubia* and *A. proteus*) used in these experiments were raised in diluted pond water from strains originally obtained from Dr. J. A. Dawson of the College of the City of New York. The centrifuge method (Heilbrunn, 1926*a*) was used for the determination of the viscosity of the protoplasm of the amoebae and the same technique was employed as that used by Heilbrunn. As in his work, an old model Bausch and Lomb centrifuge was used.

The technique for the immersion experiments is rather simple. Amoebae are placed on the bottom of a covered Stender dish (3 cm. high; diameter, 5 cm.) and are allowed to settle. The culture solution is then drained off and they are covered immediately with 20–25 cc. of the reagent of the desired concentration. As soon as possible they are transferred to centrifuge tubes which are dropped in another tall Stender dish containing the reagent. A ground-glass cover is fitted tightly on the dish to prevent evaporation of the reagent. After the desired time of immersion at room temperature (18°–25° C.), the amoebae are centrifuged and transferred to a deep depression slide and covered with a greased cover slip and examined as quickly as possible (in 10–40 seconds). In each test the viscosity of the exposed amoebae was compared with the normal or control animals from the same culture.

In the experiments on the combined effect of the fat solvent and ultraviolet radiation, the amoebae were placed in a shallow quartz dish (1½ cm. high; diameter, 7 cm.) and allowed to settle. After removing the culture solution the amoebae were covered immediately with the reagent to a depth of 1–2 mm. A quartz plate was placed over the top of the dish and the two were sealed with a thick layer of vaseline. In about 25 seconds the amoebae were exposed to the rays of a mercury vapor lamp which was the same model as used in earlier work (Heilbrunn and Daugherty, 1933) and which was loaned through the courtesy of the Hanovia Chemical and Manufacturing Company of Newark, New Jersey. The lamp was operated at about 56–58 volts and 2.5–3 amperes, which were rather constant values. The dish containing the amoebae was placed at a distance of 24.5 cm. from the arc of the lamp. During the short exposures used in the experiments there was no marked change in temperature. After an exposure of 2 minutes to the rays, the amoebae were centrifuged as noted previously.

The viscosity values for the amoebae were measured by the time in seconds required for the crystals to move through one-half of the diameter of the cell. With a few exceptions a centrifugal force of 128 times gravity was used for *A. dubia*. These relative values are roughly 1½ times as great as the absolute viscosity in terms of the viscosity of water. The plasmagel of the *A. proteus*, however, is so viscous that it was necessary to use a centrifugal force of 4,084 times gravity to move the crystals in these cells. These results are in terms of centrifuge values. The centrifuge value (Heilbrunn and Daugherty, 1932 and 1934) is defined as the number of seconds required to move the crystals of the plasmagel into half of the cell.

[2] This problem was suggested by Dr. L. V. Heilbrunn and the work was carried out under his direction. I wish to express my appreciation for his invaluable advice and criticism.

In order to determine what concentrations of the reagents should be used, many tests were made on *A. dubia* with various strengths of solutions, from the very dilute to the more concentrated ones in which the amoebae die. The concentration of the reagent used for the results recorded below was just under that in which the animals do not recover after immersion for 30 minutes. These same concentrations were used on the plasmagel of *A. proteus*, except in the case of amyl alcohol, for which a somewhat lower concentration had to be chosen.

The pH of the solutions was always between 6.5 and 7.0. The reagents used were obtained from Merck and from the Eastman Kodak Company. The absolute ethyl alcohol was redistilled. In making up the solutions water from a Kendall conductivity still was used.

RESULTS

It is common knowledge that fat solvents counteract the action of stimulating agents. The attempt was made to discover if the anesthetic effect of fat solvents could be interpreted in the light of the colloid chemical theory of stimulation as advanced above. Does the presence of a fat solvent prevent the liquefaction of the cortex? Has it any inhibiting action on the calcium release? How does the fat solvent affect the clotting reaction in the plasmasol?

In an attempt to answer these questions the following investigations on the effect of a number of alcohols and ether on both the plasmagel and the plasmasol, by themselves and in combination with ultraviolet radiation, were undertaken.

A study was first made of the effect of methyl alcohol on both the plasmagel and the plasmasol of amoebae. The first two tables show the results obtained from such a study and are examples of the type of data procured with each reagent and summarized in the other tables. The results of the effect of 1.99 molar methyl alcohol on the plasmagel of *A. proteus* are shown in Table 1. In this table the normal centrifuge value for the culture of amoebae used in each experiment is given in the third column. The centrifuge value after exposure to methyl alcohol is shown in the fourth column, and the time of immersion in the reagent is given in the fifth column. In every case the protoplasmic viscosity decreased from the normal value. This liquefaction is apparent even after immersion for 5 minutes. It will be shown later that all the alcohols used, as well as ether, cause a liquefaction of the plasmagel.

The effect of different concentrations of methyl alcohol on the plasmasol of *A. dubia* is shown in Table 2. The normal viscosity of the amoebae used in each experiment is shown in the third column. The time of immersion in the reagent is recorded in the sixth column and the viscosity after this time in the fifth column. It is apparent that in the dilute concentrations used (0.124–0.99 molar) there is a decrease in viscosity or a liquefaction which is of very short duration and which is followed by a stiffening or a gelation of the plasmasol. In the stronger concentrations (1.49 and 1.99 molar) only the gelating effect was found. These results are in accord with those obtained with ethyl and propyl alcohols and will be discussed later.

Data similar to those in Tables 1 and 2 could be given for each fat-solvent anesthetic used in the experiments, but for lack of space the summaries present the evidence found from the investigations.

Table 3 is such a summary of the effect of the fat-solvent anesthetics on the plasmagel of *A. proteus*. In this table the number of experiments for each reagent is given in the first column. The second column shows the average of the centrifuge values of the con-

trols (normal amoebae). The time of immersion in each reagent is given in the sixth column, and the average of the centrifuge values following this period of immersion are shown in the fifth column. From these results it is evident that all the alcohols and ether cause a liquefaction of the plasmagel. There is a 43.9 per cent decrease in the centrifuge value after immersion in methyl alcohol, which is accompanied by a loss of streaming and a production of inco-ordinated movements of the cell. The centrifuge value of the plasmagel decreases 49.2 per cent after immersion in ethyl alcohol, while slow inco-ordinated movement of the cell continues. Amoebae in propyl alcohol show a decrease in the centrifuge value of 66.1 per cent, while in butyl alcohol there is a decrease of only 36.6 per cent. In both of these solutions there is no movement for several minutes, and then the protoplasm seems to move in spurts. There is only a small decrease in the

TABLE 1

EFFECT OF 1.99 MOLAR METHYL ALCOHOL ON THE PLASMAGEL OF *Amoeba proteus*

Experiment Number	Temperature (Degrees Centigrade)	Normal Centrifuge Value	Centrifuge Value in Methyl Alcohol	Time Immersed in Methyl Alcohol in Minutes
1	21.5	95.0	55.0	16–23
2	21.5	105.0	70.0	21
3	21.5	105.0	75.0	23
4	25.0	110.0	50.0	20–27
5	25.0	110.0	50.0	21
6	24.0	105.0	60.0	31
7	24.0	105.0	70.0	28
8	24.5	105.0	70.0	26
9	24.5	105.0	70.0	28
10	22.5	105.0	60.0	22
11	22.5	105.0	60.0	20–26
12	24.0	110.0	40.0	29
13	24.0	110.0	40.0	24
Average	.....	105.7	59.2	.....

centrifuge value after immersion in the last two solutions; 22.2 per cent in amyl alcohol and 15.6 per cent in ether, and the amoebae continue to move. According to the modern theories of amoeboid movement (Pantin, 1923; Mast, 1926), the extent to which the plasmagel is liquefied would determine the rate of movement of the amoebae. In these experiments there is an approximate relation between the degree of liquefaction of the plasmagel and the extent to which movement is slowed or stopped.[3]

Thus it appears evident that certain fat solvents themselves produce liquefaction of the plasmagel, and they could therefore scarcely prevent the liquefaction of the plasmagel produced by stimulating agents. As far as the cortex of the cell is concerned then, there is no antagonism between stimulants and the fat solvents tested. Both following stimulation and treatment with alcohols and ether, calcium appears to be released from the plasmagel, for this shows the typical liquefaction which always follows such a release (cf. also Mazia and Clark, 1936). The released calcium diffuses rapidly in the cell interior

[3] Movement is also dependent on the fluidity of the plasmasol. Methyl, ethyl, and propyl alcohols stiffen the plasmasol (see p. 479) and thus tend to prevent amoeboid movement.

and can be assumed to initiate a clotting reaction there (see Heilbrunn and Daugherty, 1933; Heilbrunn and Mazia, 1936; Angerer, 1936).

Table 4 shows that the primary effect of certain alcohols and ether in antagonizing stimulating agents is on the plasmasol. If amoebae are immersed in one of these solutions, the plasmasol becomes more fluid. Suitable concentrations of these fat solvents,

TABLE 2

EFFECT OF METHYL ALCOHOL ON THE PLASMASOL OF *Amoeba dubia*

Experiment Number	Temperature (Degrees Centigrade)	Normal Relative Viscosity	Molarity and Percentage	Relative Viscosity in Methyl Alcohol	Time of Immersion in Methyl Alcohol
1	19.0	8	0.124=0.5	55.0	26 min.
2	20.0	8	0.124=0.5	4.0	25 sec.
				40.0	28 min.
1	20.0	8	0.249=1.0	5.0	30 sec.
				45.0	26 min.
1	20.0	8	0.374=1.5	6.0	30 sec.
				45.0	20 min.
1	19.0	5	0.623=2.5	50.0	26 min.
2	19.0	9	0.623=2.5	7.0	20 sec.
				55.0	37 min.
1	20.0	9	0.996=4.0	5.0	15 sec.
				191.4	44 min.
1	20.0	7	1.494=6.0	638.0	136 min.
2	19.0	8	1.494=6.0	638.0	135 min.
3	18.0	8	1.494=6.0	638.0	26 min.
1	20.0	6	1.99 =8.0	319.0	23 min.
2	19.5	7	1.99 =8.0	159.5	8–24 min.
3	22.5	10	1.99 =8.0	478.5	23 min.
4	22.0	10	1.99 =8.0	319.0	20 min.
5	24.0	8	1.99 =8.0	478.5	25 min.
6	22.5	8	1.99 =8.0	478.5	25 min.
7	24.5	9	1.99 =8.0	638.0	25 min.
8	20.0	9	1.99 =8.0	478.5	25 min.
9	20.0	12	1.99 =8.0	478.5	25 min.
10	21.5	8	1.99 =8.0	319.0	22–27 min.
Average		8.7		414.7	

however, prevent the clotting or gelation which follows the liquefaction of the plasmagel and the inrush of calcium to the interior of the cell.

In this table is given a summary of the effect of various alcohols and ether on the plasmasol of *A. dubia*. The centrifugal force of 128 times gravity was not sufficient to move the granules through one-half of the cell after immersion in solutions of methyl and ethyl alcohols, so that it was necessary to use the greater force or the high-speed handle of the centrifuge. In such a case this force was calculated in terms of the lower centrifugal force, on the assumption that the plasmasol behaves like a true fluid. In Table 4 the number of experiments investigated for each reagent is given in the first

column. The average values of the relative viscosity of the normal amoebae are shown in the second column. The time the amoebae were exposed to the reagent is given in the sixth column, and the average of the relative viscosity at the end of this time is presented in the fifth column.

It is evident from these results that the two lowest alcohols in the series produce a marked stiffening or gelation of the plasmasol while to a less extent propyl alcohol causes

TABLE 3

EFFECT OF FAT-SOLVENT ANESTHETICS ON THE PLASMAGEL OF *Amoeba proteus*

Number of Experiments	Normal Centrifuge Value	Molarity and Percentage	Reagent	Centrifuge Value after Immersion in Reagent	Time of Immersion in Reagent in Minutes	Percentage Increase or Decrease	Movement
13.....	105.7	1.99 M = 8%	Methyl alcohol (absolute)	59.2	16–31	43.9% decrease	No streaming of protoplasm; form of amoebae changes
10.....	100.5	1.028 M = 6%	Ethyl alcohol (absolute redistilled)	51.0	18–34	49.2% decrease	Very slow movement
8.....	94.1	0.467 M = 3.5%	Propyl alcohol (normal)	31.8	12–26	66.1% decrease	No movement for several minutes then moves in spurts
8.....	97.5	0.164 M = 1.5%	Butyl alcohol (normal)	61.8	20–27	36.6% decrease	No movement for several minutes then moves in spurts
3.....	100.0	0.066 M = 0.718%	Amyl alcohol (iso.)	65.0?	Majority go to pieces even in 12 minutes' immersion		
7.....	101.2	0.05 M = 0.5%		78.7	18–30	22.2% decrease	Moves
9.....	99.4	0.2 M = 2.06%	Ether (anhydrous distilled over sodium)	83.8	21–29	15.6% decrease	Moves

the same effect. The actual figures give an increase in viscosity of 4,730 per cent in methyl alcohol, 6,350 per cent in ethyl alcohol, and 250 per cent in propyl alcohol. Dilute solutions of each of these three alcohols were found to cause a preliminary liquefaction of very short duration in the plasmasol. In methyl and ethyl alcohols this liquefaction lasted only from 15 to about 60 seconds; in propyl alcohol, from 3 to 5 minutes.

Within the amoebae there is no streaming of the protoplasm after immersion in these three alcohols. The clear outer layers, however, were found to change the form of the

amoebae after 5 to 10 minutes of careful observation, but even this type of movement ceased after immersion for about 20 minutes in propyl alcohol.

On the other hand, the two higher alcohols and ether produce a marked liquefaction of the plasmasol, and this was evident both after short and after long periods of immersion. There was a decrease in viscosity of 59 per cent in butyl alcohol, 62 per cent in amyl alcohol, and 50–52 per cent in ether. No movement was observed in these alcohols,

TABLE 4

EFFECT OF FAT-SOLVENT ANESTHETICS ON THE PLASMASOL OF *Amoeba dubia*

Number of Experiments	Normal Relative Viscosity	Molarity and Percentage	Reagent	Relative Viscosity in Reagent	Time of Immersion in Minutes	Percentage Increase or Decrease	Movement
9.....	8.7	1.99 M = 8%	Methyl alcohol (absolute)	421.0	8–25	4,730% increase	Clear exterior, moves
9.....	8.2	1.028 M = 6%	Ethyl alcohol (absolute redistilled)	529.5	19–32	6,350% increase	After 5–10 minutes, very slowly
17.....	8.7	0.467 M = 3.5%	Propyl alcohol (normal)	30.5	19–29	250% increase	After 2–3 minutes no streaming of protoplasm; all movement ceases after 20 minutes
10.....	8.6	0.164 M = 1.5%	Butyl alcohol (normal)	6.4	3–10	25.5% decrease	No movement
				4.1	20–29	59% decrease	.....
10.....	8.7	0.066 M = 0.718%	Amyl alcohol (iso.)	6.8	1– 8	21.8% decrease	No movement
				3.2	15–23	62% decrease	.....
9.....	8.0	0.2 M = 2.06%	Ether (anhydrous)	3.7	2.5– 5	52.8% decrease	Moves
				4.0	18–28	50% decrease	.....

and after 15–30 minutes the granules settled to such an extent that it was necessary to roll the tubes containing the amoebae and the reagent during the whole time of each experiment in order to obtain centrifuge tests (for a discussion of this technique see Heilbrunn and Daugherty, 1934). Occasionally the granules settled within 25–30 minutes in ether; and while after this time there was no streaming of the internal protoplasm, there was a definite change in the form of the amoebae.

It is surprising to note that low concentrations of these higher alcohols and ether were found to cause first a liquefaction which was then followed by a stiffening or gelation of the plasmasol. In 1 per cent of ether there is a decrease in viscosity of the plasmasol of approximately 50 per cent, and this is then followed by an increase in viscosity to a value of 2 or 3 times that of the normal.

In these results it may be noted that the liquefying effect of the fat-solvent anesthetic which would tend to prevent the clotting reaction of stimulation is produced only by the higher alcohols and ether. The lower alcohols and low concentrations of all the fat solvents used do not prevent clotting of the plasmasol, and they may even initiate it. In the lower alcohols there is also presumably a dehydrating action on the proteins of the protoplasm. As far as effects on viscosity are concerned, the lower alcohols and low concentrations of the higher alcohols and ether act in somewhat the same manner as stimulating agents. Probably calcium is released from the plasmagel and diffuses into the interior of the cell, producing the clotting reaction. Thus the theory outlined previously can provide an explanation for the stimulating action of dilute fat solvents.

Up to this point the effect of various alcohols and ether alone has been considered. From the results obtained it seems logical to assume that the higher alcohols and ether, inasmuch as they tend to liquefy the plasmasol, would exert an antagonistic effect on the gelation or clotting of the plasmasol produced by stimulating agents. In the following experiments an attempt is made to study the effect of several alcohols and ether on the stimulating action of ultraviolet radiation.

The results of such an investigation on the plasmasol are summarized in Table 5. As before, the number of experiments is given in the first column. In the second column is shown the relative viscosity of the control or normal amoebae. A second control, a measurement of the viscosity after exposure to ultraviolet radiation alone, seemed unnecessary after several experiments as the increases in viscosity averaged the same as those previously reported (Heilbrunn and Daugherty, 1933 [Table 1]). This work showed that an exposure for two minutes under the same conditions as those used in the present experiment produced a 500 per cent increase in the viscosity of the plasmasol. In Table 5 the time after radiation at which the measurements were made is given in the sixth column and the viscosity values after exposure to both the reagent and the ultraviolet radiation are shown in the fifth column. There is an increase of 2,560 per cent in the viscosity of the amoebae immersed in methyl alcohol 6–25 minutes after irradiation. No movement was observed in these animals. In the amoebae immersed in ethyl alcohol there is first a slight decrease of 21.9 per cent in the viscosity, $2\frac{1}{2}$–6 minutes after radiation, while after a longer time (6–28 minutes) it increases 550 per cent from the normal value. In this solution the amoebae show no protoplasmic streaming, but the outer clear layers continue to move. Only about 50 per cent of the amoebae recover from the combined treatment of these alcohols and ultraviolet radiation. After exposure to propyl alcohol and radiation there is a 317 per cent increase in the viscosity of the plasmasol. These amoebae continue to move for about 25 minutes, and about 75 per cent recover.

On the other hand, this table shows clearly that the effect of the ultraviolet radiation is completely inhibited by the higher alcohols and ether. Now, instead of an increased viscosity of the plasmasol, such as always occurs with ultraviolet radiation alone, there is a decrease in its viscosity. In the presence of these fat solvents the ultraviolet radiation is completely without effect in so far as the viscosity of the plasmasol is concerned. After the combined effect of one of the alcohols or ether and the ultraviolet radiation there is the following decrease in the viscosity of the plasmasol: 45 per cent in butyl alcohol, 10–27 minutes after radiation; 33.3 per cent in amyl alcohol, 2–15 minutes after radiation, after which time the granules settled; 48 per cent in ether, 2–27 minutes after radiation. No movement was observed after exposure to butyl alcohol and radiation, and there was only a very slow movement after radiation and amyl alcohol. In ether plus the radiation the amoebae were inactive for 5–10 minutes, after which they began

to move very slowly. Recovery of the amoebae in the alcohols after exposure to the radiation was about 50 per cent; recovery in ether after exposure to radiation was about 90 per cent.

A few experiments on the combined effect of the reagent and ultraviolet stimulation were made on the plasmagel of *A. proteus*. A marked liquefaction occurred which was similar to the effect produced by either agent alone. As this result was to be expected, no extensive series of experiments was planned.

TABLE 5

COMBINED EFFECT OF THE FAT-SOLVENT ANESTHETIC AND ULTRAVIOLET STIMULATION ON THE PLASMASOL OF *Amoeba dubia*

Number of Experiments	Normal Relative Viscosity	Molarity and Percentage	Reagent	Viscosity in Reagent and after Ultraviolet Radiation for 2 Minutes	Time after Radiation in Minutes	Percentage Increase or Decrease	Movement
9.....	8.1	1.99 M = 8%	Methyl alcohol (absolute)	233	6–25	2,560% increase	No movement; 50% recover
8.....	8.2	1.028 M = 6%	Ethyl alcohol (absolute redistilled)	6.4	2½– 6	21.9% decrease	Clear exterior changes form 50% recover
				53.3	6–28	550% increase	
9.....	9.7	0.467 M = 3.5%	Propyl alcohol (normal)	30.5	5–32	317% increase	No movement after 25 minutes' immersion; 75% recover
7.....	9.4	0.164 M = 1.5%	Butyl alcohol (normal)	5.1	10–27	45% decrease	No movement; 50% recover
11.....	9.0	0.066 M = 0.718%	Amyl alcohol (iso.)	6.0	2–15	33.3% decrease	No movement for 2 or 3 minutes, then slowly; 50% recover
9.....	8.4	0.2 M = 2.06%	Ether (anhydrous)	4.3	2– 8	48.4% decrease	No movement for 5–10 minutes, then slowly; 90% recover
				4.2	18–27		

DISCUSSION

It has been suggested (Heilbrunn, 1928 [chap. vi]; Heilbrunn and Daugherty, 1933; Heilbrunn and Mazia, 1936) that stimulation involves a gelation of the main mass of protoplasm similar in some respects to the coagulation of blood. This colloidal theory of stimulation postulates the following series of changes: (1) a liquefaction of the cortex of the cell which releases calcium and thus increases the permeability of the membrane to water-soluble substances; (2) a transitory liquefaction of the plasmasol; and (3) a gelation of the plasmasol.

The work on ultraviolet radiation and mechanical pressure has shown that these agents produce the first change—that of liquefaction of the cortex or plasmagel of amoeba. And the idea of the release of calcium is consistent with the known effect of various stimulants on living material. Heilbrunn and Daugherty (1933), while not able to show the direct release of calcium, did prove that in the presence of oxalate the action of ultraviolet radiation was unable to cause the clotting of the plasmasol. Nadson and Gleichgerwicht (1928) first showed that ultraviolet radiation produces crystals of calcium oxalate in *Elodea* cells. The most convincing evidence is presented by Mazia and Clark (1936). Various stimulants such as the electric current, ultraviolet irradiation, mechanical pressure, heat, and hypertonic salt solutions were found to produce crystals which were definitely proved to be crystals of calcium oxalate in the vacuoles of *Elodea* cells.

It is also evident that when calcium is removed from the surface membrane there is an increase in the permeability of the cell. This is shown in the older literature (Stiles, 1924). McCutcheon and Lucké (1928) also found a marked increase in the permeability to water of *Arbacia* eggs in dextrose solutions from which calcium or magnesium had been removed. Addition of either of these ions caused a decrease in the permeability to water. Weber (1932), working on the plasmolysis of *Spirogyra* and onion cells, found that when calcium is removed urea enters the cells more quickly and the cell swells.

Any theory of stimulation should be a theory of anesthesia. If it is true that stimulating agents produce the effects outlined above, and if these effects are essential to response, then it should also be true that in some way anesthetics prevent the chain of events as outlined.

In so far as the plasmagel is concerned, fat-solvent anesthetics act in the same way as stimulating agents. There is obviously no antagonism. However, there is antagonism in the case of the plasmasol, for whereas stimulation causes gelation or clotting, the higher alcohols and ether prevent the gelation, if they are present in sufficient concentrations. It thus seems clear that the fat-solvent anesthetics—at any rate those which were studied—inhibit the action of stimulating agents by inhibiting the clotting of the plasma sol which normally follows stimulation. This is in line with the observation of Heilbrunn (1934*a*) that the surface precipitation reaction, which is fundamentally the same as the clotting reaction, is prevented by fat solvents.

Of the alcohols, only the higher ones act in this way. The lower alcohols are without effect. Moreover, the higher alcohols and ether must be present in sufficient concentrations. In low concentrations of these reagents there is no prevention of the gelation produced by stimulating agents. Indeed, such low concentrations themselves cause liquefaction of the plasmagel, and this is followed by a transient liquefaction and then by a great increase in the viscosity of the interior plasmasol. In other words—and this seems very important—low concentrations of these fat-solvent anesthetics act like stimulating agents. Both stimulating agent and anesthetics produce the same changes in the plasmagel. According to the colloid chemical theory, calcium is released; this enters the interior and tends to produce liquefaction, followed by gelation. Now, in the presence of a high-enough concentration of fat solvent, the gelation is prevented; in lower concentrations it is not prevented. Thus, it is clear that low concentrations of fat-solvent anesthetics behave in the same manner as stimulating agents. The explanation for this behavior now seems apparent. It furnishes a logical interpretation of what has been one of the outstanding problems of the theory of anesthesia.

SUMMARY

1. Methyl, ethyl, propyl, butyl, amyl alcohols, and ether in concentrations just below lethal cause a liquefaction of the plasmagel of amoeba.

2. In the same concentrations the lower alcohols, except for perhaps a transitory liquefaction, produce only a gelation of the plasmasol; the higher alcohols and ether cause a liquefaction of the plasmasol.

3. More dilute solutions of the higher alcohols and ether produce first a liquefaction and then a gelation of the plasmasol.

4. Study of the combined effect of these fat solvents and ultraviolet radiation shows that they are not antagonists in so far as the plasmagel or cortical protoplasm of amoeba is concerned.

5. The higher alcohols and ether prevent the typical clotting reaction of stimulation.

6. It is concluded, therefore, that the higher alcohols and ether produce their anesthetic effect by preventing the gelation of the plasmasol.

7. The effects of dilute solutions of these alcohols and ether resemble the effects of stimulating agents. The explanation of this phenomenon and its importance for the theory of anesthesia are discussed.

8. These results as a whole strongly support the colloid chemical theory of stimulation as developed by Heilbrunn, 1928; Heilbrunn and Daugherty, 1933; and Heilbrunn and Mazia, 1936.

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